Production and Characterisation of Recombinant *Plasmodium vivax* Merozoite Surface Protein-1_{19} Expressed in *Lactococcus lactis*

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Production and Characterisation of Recombinant *Plasmodium vivax* Merozoite Surface Protein-1_{19} Expressed in *Lactococcus lactis*

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Dissertation submitted to Sister Nivedita University, in partial fulfilment of the requirement for M.Sc. degree in Biotechnology

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CERTIFICATE

This is to certify that the thesis entitled: "**Production and Characterisation of Recombinant *Plasmodium vivax* Merozoite Surface Protein-1_{19} Expressed in *Lactococcus lactis***" submitted in partial fulfilment of the requirement for the award of the degree of Master of Science in Biotechnology to Sister Nivedita University, Kolkata is a faithful record of bonafide and original research work carried out by **Ms. Tiasha Ghosh** bearing Enrolment no: 2322210002006 under my guidance and supervision from 15/01/2025 to 25/05/2025. Further, it is also to certify that the above-said work has not been previously submitted for the award of any degree, diploma, or fellowship in any Indian or foreign University. The assistance and help received by her from various sources during the course of investigation have been duly acknowledged.

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DECLARATION BY THE STUDENT

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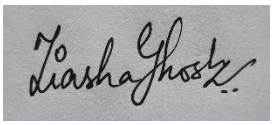

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TO WHOM IT MAY CONCERN

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Abbreviations

1. *P. falciparum – Plasmodium falciparum*
2. *P. vivax – Plasmodium vivax*
3. pf – *Plasmodium falciparum*
4. pv – *Plasmodium vivax*
5. *L. lactis – Lactococcus lactis*
6. Spz – Sporozoite
7. Mrz – Merozoite
8. pRBCs – Packed Red Blood Cells
9. MSP – Merozoite Surface protein
10. His – Histidine
11. kDa – Kilo Dalton
12. M – Molarity
13. N – Normality
14. RBC – Red Blood Cells
15. LB – Luria Broth
16. LAB – Lactic Acid Bacteria
17. *E. coli – Escherichia coli*
18. GRAS – Generally Recognised as Safe
19. LPS – Lipopolysaccharide
20. SOC – Super Optimal Catabolite
21. Cryo – Cryopreservation
22. ERM – Erythromycin
23. TAE – Tris-acetate-EDTA
24. DNA – Deoxyribonucleic Acid
25. PBST - Phosphate-Buffered Saline with Tween-20
26. ELISA – Enzyme-Linked Immunosorbent Assay
27. PBS - Phosphate-Buffered Saline
28. HRP – Horse Radish Peroxidase
29. TMB - 3,3',5,5'-Tetramethylbenzidine
30. OD – Optical Density
31. RPM – Revolutions Per Minute
32. CV – Column Volume
33. Ni-NTA – Nickel Nitrilotriacetic Acid
34. SDS PAGE – Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
35. PVDF – Polyvinylidene Fluoride

36. ECL – Enhanced chemiluminescence
37. TBST – Tris-buffered saline with Tween-20
38. $AmSO_4$ – Ammonium Sulphate
39. NaAc –Sodium Acetate
40. KH_2PO_4 – Potassium Phosphate
41. Tris-HCl - Tris(hydroxymethyl)aminomethane hydrochloride
42. PBS – Phosphate buffer saline
43. NaCl – Sodium Chloride
44. EDTA - Ethylenediaminetetraacetic Acid

Abstract

Malaria continues to pose a significant global health threat, with *Plasmodium vivax* responsible for a substantial proportion of cases outside sub-Saharan Africa. Despite ongoing vaccine development efforts, the unique biological complexity of *P. vivax*, including its dormant liver-stage hypnozoites and reticulocyte-specific invasion mechanisms, has rendered the formulation of an effective vaccine particularly challenging. Among the blood-stage antigens, Merozoite Surface Protein-1 (MSP-1) has emerged as a promising candidate, particularly its conserved 19-kDa C-terminal fragment, MSP-1_{19}, known to induce strong and potentially protective humoral immune responses.

This study aimed to express, purify, and characterise the recombinant PvMSP-1_{19} protein in *Lactococcus lactis*, a Gram-positive, generally regarded as safe (GRAS) bacterial host. The selection of *L. lactis* over traditional hosts like *E. coli* was driven by its superior expression system for complex antigens. A codon-optimized construct encoding PvMSP-1_{19} with a C-terminal His-tag was cloned into a lactate-inducible expression vector and successfully transformed into *L. lactis*. Following screening, high-yield clones were selected, and small-scale fermentations were performed. Recombinant protein expression was validated using SDS-PAGE and western blotting. The protein was subsequently purified using Chromatographic techniques. Structural confirmation and immunoreactivity were assessed through Sera ELISA using anti-human IgG antibodies.

This study confirmed successful expression and purification of immunogenic PvMSP-1_{19}, demonstrating its potential as a viable subunit vaccine candidate against *P. vivax* malaria. This work establishes a proof-of-concept for the use of *L. lactis* as a host for malaria vaccine antigen production and contributes to the broader pursuit of safer, cost-effective, and scalable vaccine development strategies targeting blood-stage malaria antigens.

Keywords

1. *Plasmodium vivax*
2. MSP-1_{19}
3. Merozoite surface protein
4. Blood-stage malaria vaccine
5. Merozoites
6. *Lactococcus lactis*
7. Recombinant protein expression
8. His-tag purification
9. SDS-PAGE
10. IMAC
11. IEC
12. ELISA
13. Vaccine candidate
14. Asexual blood stage
15. Endotoxin-free expression
16. Host-vector interaction

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1. Introduction

"Malaria eradication has been compared with high-mountain climbing: more obstacles appear as one approaches the summit, and at that stage, every step demands an increased effort." - Bruce Chawatt L.J. (1965)

1.1. The disease:

With high rates of morbidity and mortality, malaria is one of the most important infectious illnesses in the world, especially in tropical and subtropical areas. An estimated 3.2 billion individuals spanning 95 different countries and territories are at risk of acquiring the mosquito-borne disease (1). Female *Anopheles* mosquitoes transmit malaria and disproportionately affect the world's poorest communities. Eight protozoan parasite species, including the most prevalent ones, *P. falciparum* and *P. vivax* of the *Plasmodium* genus, can infect people and cause malaria. *P. falciparum* is mostly to blame for morbidity and mortality (2).

Sporozoites are introduced into the human host by biting a female *Anopheles* mosquito. These sporozoites develop as they move to the liver and eventually the bloodstream. Following an infected individual's blood meal, the malarial gametocytes enter the mosquito's midgut and develop into female macrogametes and male microgametes. The zygote produced by their union develops into an ookinete, passing through the mosquito's intestinal wall and becoming a circular oocyst. The sporozoites grow from sporoblasts, germinal cells found inside the oocyst. After emerging from the oocysts, the sporozoites move to the salivary gland and infect human hosts when the mosquito feeds on their blood (3).

Plasmodium falciparum is a deadly parasite that causes serious illness and most malarial deaths, particularly in sub-Saharan Africa. Although *Plasmodium vivax* has a far wider geographic range than *P. falciparum*, it usually produces milder illnesses (4). The second

most common species of human malaria, *Plasmodium vivax*, puts about 35% of the world's population at risk of contracting the disease with severe and fatal outcomes (4,5).

The most obvious propensity of *P. vivax* is to trigger relapses weeks to months after a primary infection by triggering dormant liver-stage parasites called hypnozoites. The hypnozoite is a reservoir of infection made up of these dormant liver-stage infections. These communities receive new blood infections and clinical attacks from that reservoir, as well as chances for further transmission. The hypnozoite reservoir competently expands its native geographic range into temperate zones by extending clinical attacks over seasons unsuitable to anopheline mosquitoes (4,5).

1.2. Malaria, a consequence of poverty:

Malaria is a serious global public health issue that has been estimated to kill 627,000 people in 2020 and affect 241 million people (11). Nevertheless, the majority of nations experiencing continuous malaria transmission are low-income nations, and while the International Health Regulations were mainly implemented to guarantee national capacity building to accomplish the shared objective of global health security, many low-income countries are unable to put the essential core capacities into place (12,13). Complex interactions and feedback cycles between humans, mosquitoes, parasites, their habitats, healthcare systems, and the execution of policies at a particular time are what mediate the transmission and management of malaria (8,9). Prolonged exposure has caused parasites and mosquitoes to be resistant to drugs and insecticides, respectively, making the elimination of malaria a critical challenge (12).

1.3. Therapeutics:

Clinically, the pre-erythrocytic phase of the parasite life cycle is quiet. The blood stage is the cause of malaria's symptoms, which might include fever, chills, headache, muscle aches, altered consciousness, nausea, vomiting, diarrhea, exhaustion, and respiratory

distress. Usually categorized as asymptomatic, uncomplicated, symptomatic, or severe, malaria can quickly worsen if it is not identified and treated right away (22).

Three strategies are used to combat malaria parasites: immunization, seasonal prevention, and antimalarial medications (10). Both the life cycle and the illness process depend heavily on *Plasmodium's* capacity to identify and subsequently infiltrate hepatocytes, or red blood cells. It is widely known that *Plasmodium* sporozoites move through Kupffer cells and many hepatocytes during the pre-erythrocytic stage before ultimately infecting a hepatocyte. As a result, antigens found on the parasite's surface or, more precisely, in its apical organelles during the phase have been proposed as a target for a better knowledge of the *Plasmodium* lifecycle and, perhaps, for application in vaccines (6,7).

The significant variability in the primary proteins expressed during *Plasmodium* sporozoite (Spz) and merozoite (Mrz) invasion, as well as the alternating usage of invasion routes, is one of the primary challenges for developing an anti-malarial vaccine. Alternatives for covering the wide variety and variety of proteins employed during invasion must be considered because of these two evasion mechanisms (polymorphism and various invasion routes). Reducing the total number of possibilities to be examined to further this search can be accomplished with the help of genetic diversity and evolutionary force analysis (drift, selection, and recombination) (14, 15, 16).

1.4. Vaccine Candidate Selection:

A possible malaria vaccine candidate should focus on three stages. The pre-erythrocytic stage is the initial target of vaccine development. During this time, sporozoites enter the bloodstream and infect hepatocytes, causing them to go through schizogony, a stage of vigorous multiplication that comes before red blood cell (RBC) invasion. The parasite's blood stage is the second target for the design of malaria vaccine candidates. Evidence that people who have recurrent malaria infections in endemic areas develop some degree of protective immunity—a condition in which there is immune-controlled RBC invasion—

provides the impetus for the development of such vaccine candidates. Gametocytes, or sexual parasite forms, are the third target of the malaria vaccine candidate. Through the host's immune system, malaria transmission-blocking vaccines (TBVs) aim to stop the spread of parasites from humans to the mosquito vector.

After a century of research, we still lack a highly potent malaria vaccine that can produce long-lasting protective immunity. The intricacy of the malaria parasite's life cycle, the mostly stage-specific expression of immune response targets, and the parasite's capacity to elude and alter the human immune response are some of the parasite-specific causes of this (23). Some primary malaria medicines include antimalarial drugs like chloroquine, artemisinin-based combination therapies (ACTs), and doxycycline.

Three strategic objectives are outlined in the WHO's preferred product characteristics (PPC) document for malaria vaccines: Malaria vaccinations that (i) prevent human blood-stage infection at the individual level, (ii) lower malaria morbidity and mortality in susceptible individuals in malaria-endemic areas, and (iii) decrease parasite transmission, which significantly lowers the prevalence of human infection in the community (24,25).

One of the primary challenges for developing an anti-malarial vaccine is the significant variability in the primary proteins expressed during *Plasmodium* sporozoite (Spz) and merozoite (Mrz) invasion, as well as the alternating usage of invasion routes (14,15). Depending on the sites and stages of infection in malaria, potential vaccine candidates are selected.

In a recent study, it was shown that a portion of PvMSP-1 may represent a crucial parasite adhesion protein in the binding of *P. vivax* merozoites to erythrocytes. Here, the work is done on a construct, namely, PvMSP-1_{19} - His, understanding its role as a potential protein in producing immunogenicity.

2. Review of Literature

The intricacy of the malaria parasite's life cycle, mostly the stage-specific expression of immune response targets, and the parasite's capacity to avoid and alter the human immune response are some of the parasite-specific aspects that contribute still why we do not have an effective malaria vaccine (26). By 2030, the updated Malaria Vaccine Technology Roadmap seeks to develop vaccinations with at least a 75% efficacy in preventing P. falciparum clinical malaria and immunity for a minimum of two years (27).

Most vaccine candidates in clinical research fall into one of two general categories: subunit or whole-parasite vaccinations. To allow for the highest possible number of proteins (some of which are conserved across strains of parasites) that can be used by the immune system and to ensure immunization against a broad range of proteins, the whole parasite has been incorporated into the vaccine. This strategy is believed to reduce the influence of variations in any one parasite protein, which has been shown to have a detrimental effect on the effectiveness of subunit vaccination candidates (28,29). Sub-unit vaccines, which include a single parasite protein eliciting an antibody response, comprise most of the malaria vaccines currently in development. The vaccine's efficacy can be affected by these specific proteins across *Plasmodium* strains, which may manifest as diminished protection against parasite strains compatible with the vaccine (28).

The developed or ongoing trials of vaccine candidates target certain stages of the infection. It either includes individual stages like the liver stage, asexual blood stage, and sexual stage or targets multiple stages at once.

2.1. *Plasmodium* Life Cycle:

Malaria infection starts when a female *Anopheles* mosquito injects sporozoites into a human's skin while feeding on blood. After actively entering the peripheral circulation, the sporozoites move to the liver and infiltrate hepatocytes. Sporozoites divide asexually within hepatocytes to produce thousands of merozoites as depicted in **Figure 2.1**.

The asexual blood stage is initiated by the release of the merozoites into the circulation after maturation. The merozoites invade red blood cells (RBCs) rapidly and pass through three developmental stages: ring, trophozoite, and schizont. Schizont rupture releases new merozoites, extending the erythrocytic cycle and inducing symptoms such as fever, anemia, and others. Approximately 0.1–5% of the asexual parasites mature to become their sexual forms (gametocytes).

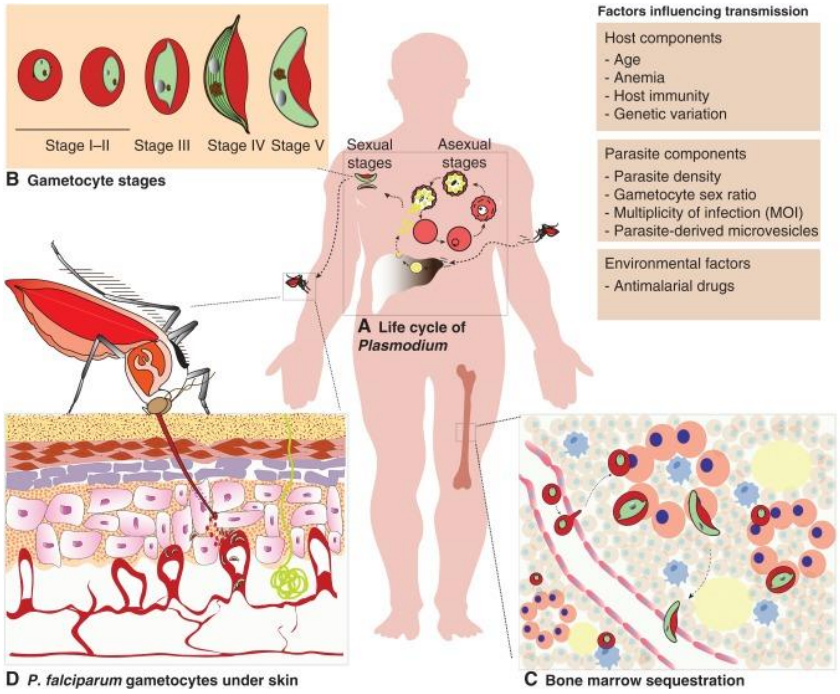


Fig 2.1: The life cycle of *Plasmodium* spp in the human host and mosquito vector is shown schematically (53)**.**

There are five different morphological stages (I–V) that gametocyte development goes through:

- Stages I–IV: immature forms that are mostly confined to the extravascular space of the bone marrow. Early gametocytes stick to the erythroblastic islands and the bone marrow sinusoids' endothelial walls.
- Stage V: Mature gametocytes regain their peripheral circulation and become deformable, primarily settling in the skin's subdermal microcapillaries.

When a mosquito feeds on a human, it consumes the mature male and female gametocytes. Issues in the mosquito's midgut are brought on by environmental stimuli like temperature decreases, pH rises, and the presence of xanthurenic acid. Male gametocytes produce eight motile microgametes (exflagellation). female gametocytes to produce a macrogamete. The result of fertilization is a diploid zygote. The zygote forms an invading active ookinete that invades the mosquito's midgut epithelium. On the exterior of the midgut wall, ookinetes mature into oocysts. Oocysts mature and release thousands of sporozoites in 11–16 days. **Figure 2.2** depicts the phases of *Plasmodium*, the liver and blood stages in the human host, and the sexual cycle in the mosquito vector.

2.2. Liver Stage Vaccines:

RTS, S/AS01 (Mosquirix), the only vaccine candidate that has completed testing in phase III trials, was recently recommended for the use of children in sub-Saharan Africa at increased risk of P. falciparum infection, though it has only modest efficacy against clinical malaria (30).

2.3. Asexual Blood stage vaccines:

The goal of vaccines that target the malaria parasite's asexual blood stage is to lower the parasite burden and stop clinical illness. A blood-stage vaccination might be an effective way to lower parasitemia and the morbidity and mortality of malaria. Most vaccine

candidates try to do this by generating antibodies that prevent the merozoite from invading red blood cells, stop the pRBCs from adhering to the vasculature in vital organs, and stimulate phagocytosis of pRBCs. The ability of the parasite to identify and infiltrate reticulocytes is crucial to the pathophysiology of *P. vivax* infections. This intricate process relies on several highly precise and sequential ligand-receptor interactions between merozoites and the host erythrocyte surface proteins. Polymorphic vaccine antigens that are immunologically different and antigen production difficulties are only a couple of the obstacles researchers have faced when designing blood-stage vaccines. The pre-erythrocytic stage and the development of vaccines that prevent transmission are similarly affected by some of these issues (20,25).

First, immunity against blood-stage antigens can be developed naturally through exposure to *P. vivax* infection; second, protection against *P. vivax* infection is enhanced by immunization against blood-stage antigens; and third, the inhibitory effect of antibodies against erythrocyte binding and serological responses to blood-stage antigens both increase with age, indicating a boosting effect brought on by repeated exposure through recurrent infection. These results point to the blood-stage antigen's immunogenicity (25,52).

A blood-stage vaccine may also lessen gametocytemia in the host, which would obliquely lessen transmission. Furthermore, from a biological standpoint, antibodies that target merozoite antigens have a tiny window of opportunity to neutralize the parasite before it invades the red blood cell (20,21,25). Some already established vaccine candidates that target the blood stage as of April’25 are as follows:

2.3.1. RH5.1/Matrix-M

- Target Antigen: Reticulocyte-binding protein homolog 5 (RH5)
- Developer: University of Oxford and Serum Institute of India.
- Clinical Status: Phase IIb trials.

- Efficacy: Demonstrated 55% efficacy against clinical malaria in a delayed third-dose regimen and up to 80% efficacy against high parasite density.

2.3.2. MSP 3 LSP

- Target Antigen: Merozoite Surface Protein 3 (MSP 3).
- Developer: African Malaria Network Trust (AMANET).
- Clinical Status: Undergoing safety testing in children in Burkina Faso and Tanzania.

2.3.3. GMZ2

- Target Antigens: Glutamate-rich protein (GLURP) and MSP 3.
- Developer: African Malaria Network Trust (AMANET).
- Clinical Status: Initiated Phase 1b adult study in Gabon.

2.3.4. P27A Vaccine

- Target Antigen: Synthetic peptide from *Plasmodium falciparum* trophozoite exported protein 1 (TEX1)
- Clinical Status: Phase I trials.

2.4. Transmission-Blocking Vaccines (Sexual stage):

Aiming to produce antibodies that stop the parasite from growing within the mosquito, transmission-blocking vaccinations usually incorporate surface antigens from the parasite's sexual and mosquito stages. This stops the parasite from spreading. With enough vaccination coverage, these vaccines seek to create community-based immunity rather than directly benefiting the recipient. Antigen manufacturing, vaccine candidates' subpar immunogenicity, and the quick decline of antibodies after vaccination have been the primary obstacles to this vaccine strategy. There have only been findings from clinical trials involving the sub-unit vaccine candidates Pfs230 (gametocyte/gamete antigen) and Pfs25 (a mosquito-expressed post-fertilization antigen) (25,27).

Table 2.1 (below) displays potential malaria vaccine candidates (collected from various papers), also known as transmission-blocking vaccines (TBVs), which work to prevent the parasite from spreading from humans to mosquitoes instead of directly preventing illness.

Table 2.1: Transmission-blocking vaccine candidates.

Vaccine Candidate	Target Stage	Species	Status
Pfs25	Female gamete/post-fertilization	*P. falciparum*	Phase I
Pfs230	Male gamete	*P. falciparum*	Phase I/II
Pvs25	Female gamete	*P. vivax*	Preclinical
HAP2	Gamete fusion	*P. falciparum/vivax*	Preclinical

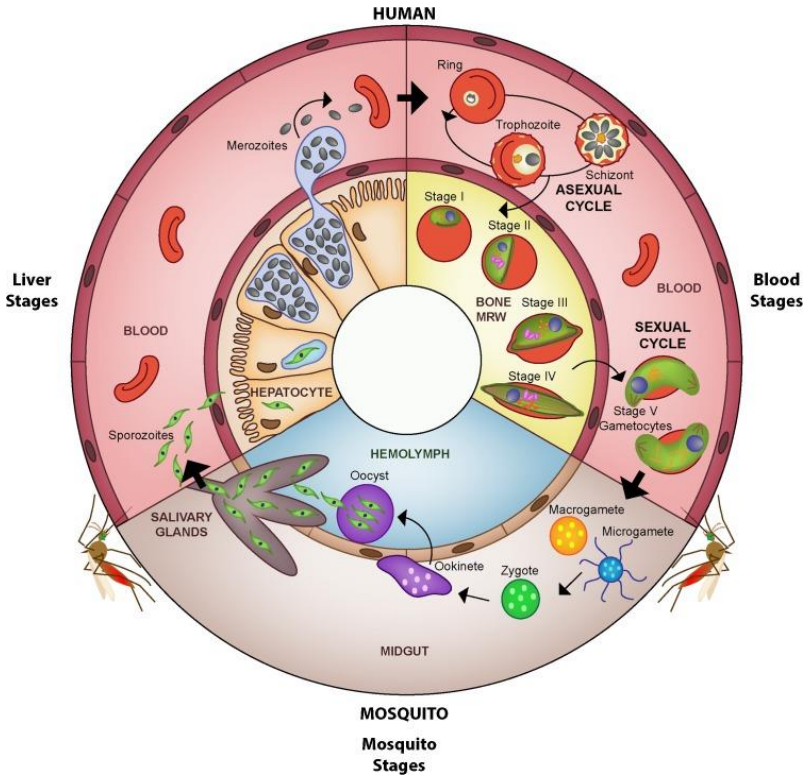


Fig. 2.2: Life cycle of *Plasmodium* species, showing the liver and blood stages in the human host and the sexual cycle in the mosquito vector (50)**.**

2.5. Update on *P. vivax:*

P. vivax is categorized as a neglected tropical illness, particularly concerning the development of species-specific vaccines and anti-relapse treatments as it has a complex life cycle with multiple stages of differentiation. Sporozoites travel through the liver, infiltrate hepatocytes, and mature into thousands of merozoites, which are eventually discharged into the bloodstream when the hepatocyte ruptures. Some parasites, known as relapse infections, stop the growth of hepatocytes from remaining dormant for a short time called hypnozoites, which allows for numerous consecutive clinical relapses and possible transmission from a single infection. Within 48 hours after being released into the bloodstream, merozoites infect reticulocytes and start the cyclical asexual evolution from rings to trophozoites to merozoites. To restart the asexual cycle, the matured infected reticulocyte bursts, releasing 8–32 fresh blood-stage merozoites into the blood. As a result of the toxins generated throughout this developmental cycle, *P. vivax* malaria is characterized by a tertian pattern of recurrent fever, or paroxysm (18). A schematic representation of the *Plasmodium vivax* life cycle is shown in **Figure 2.3**.

The parasite reproduces sexually in the mosquito to create sporozoites, which enter the mosquito's salivary glands, where they become infectious, and are then injected into a new host during a blood meal. Given the intricate life cycle of the parasite and its insufficient capacity to stop transmission and relapse infections, the development of vaccines ought to be a key component of the overall malaria control strategy creating a challenge for performing a reliable Controlled Human Malaria Infection (CHMI) investigation have increased with *P. vivax*, especially because there is no continuous parasite culture technique. The time needed to produce successful vaccines and medications would be greatly impacted by discovering culture conditions that could sustain asexual propagation while retaining prolific gametocyte production. Thus, a thorough understanding of the biology of this parasite has long been impeded by the absence of a continuous in vitro culture system (31,19). *P. vivax* populations exhibit

extraordinary genetic and phenotypic variety, including differences in their local vector adaptation and periodicity patterns (recurrent fevers associated with parasite release from red blood cells). The macaque parasite *Plasmodium cynomolgi* seems to be the closest species to *P. vivax* in Southeast Asia based on their similar biological characteristics (17).

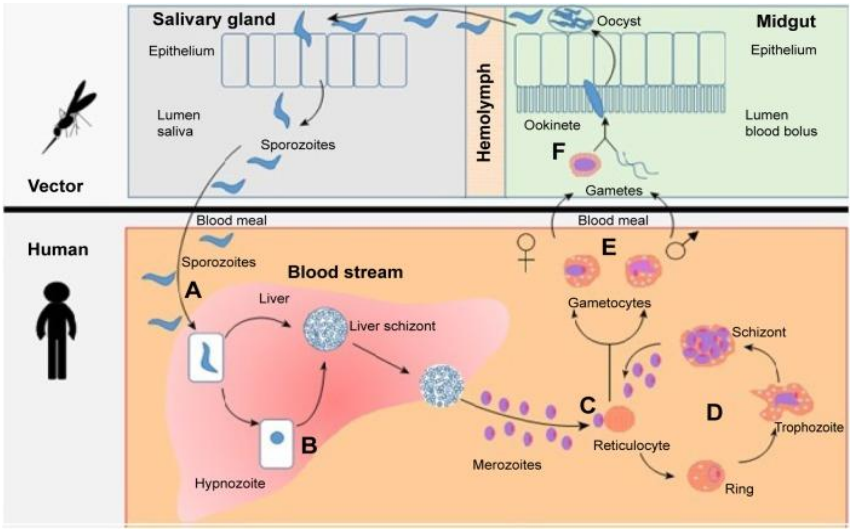


Fig 2.3: Schematic representation of the *Plasmodium vivax* life cycle in the human host and mosquito vector (51)**.**

Recently, numerous teams have used enriched reticulocytes from cord blood to establish short-term *P. vivax* cultures for invasion inhibition experiments successfully. However, the routine use of such tests is limited to endemic areas because these methods still depend on the availability of fresh *P. vivax* isolates from patients. Furthermore, access to blood samples from *P. vivax* patients is necessary for producing infected mosquitoes for sporozoite- and/or transmission-stage investigations without a *P. vivax* blood-stage culture method that can also yield gametocytes. A breakthrough in *P. vivax* research and vaccine development will depend on closing this gap (31,32,33,34).

2.6. PvMSP-1_{19} - His:

Developing a vaccine requires determining which antigens serve as targets for naturally occurring antibodies. Among the 3 targets of infection for the production of malaria vaccine, the asexual blood stage antigens intended to produce inhibitory antibodies against the circulating merozoites, stopping them from infecting red blood cells, are one of the three key targets for a malaria vaccine.

One such blood-stage candidate is merozoite surface protein-1 (MSP-1). MSP-1 is expressed as a large, complex precursor (~200kDa) on the merozoite surface. MSP-1, which is mostly expressed during the trophozoite stage, is proteolytically divided into four main fragments at the time of schizogony: MSP-1_{83}, MSP-1_{30}, MSP-1_{38}, and MSP-1_{42} (43). The 42kDa of merozoite surface protein-1 (MSP-1_{42}) is broken down by proteolysis during merozoite invasion, resulting in a 33kDa soluble fragment (MSP-1_{33}) and a 19kDa fragment (MSP-1_{19}), which stays on the merozoite surface by glycosylphosphatidylinositol (GPI) moiety and is transported into freshly invaded RBCs. (40,41).

Fig. 2.4: Schematic representation of the recombinant gene construct used in the study for expression in *Lactococcus lactis.*

The construct **(Figure 2.4)** includes a lactate-inducible promoter (p170), a multiple cloning site (MCS) with BamHI and SalI restriction sites, and the gene encoding the target protein with a C-terminal 6xHis tag for purification. The expression vector used is pSS1.

2.6.1. Structure and Origin of MSP-1_{19}

- MSP-1 is initially synthesized as a large precursor (~190kDa).
- It is cleaved during schizont rupture into several fragments.
- The C-terminal 19-kDa fragment (MSP-1_{19}) remains attached to the merozoite surface via a glycosylphosphatidylinositol (GPI) anchor.
- MSP-1_{19} consists of two epidermal growth factor (EGF)-like domains, which are structurally stable and highly conserved. **Figure 2.5** depicts a reference 3D PvMSP-1_{19} model from PMID: 22445906.

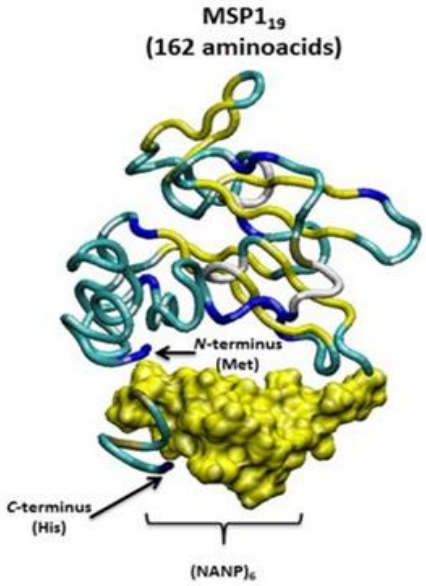


Fig. 2.5: A reference 3D PvMSP-1_{19} model was obtained (49).

2.7. PvMSP-1_{19} as a strong immunogen:

A study highlights the strong immunogenicity of the conserved C-terminal region of PvMSP-1 and the notable immune response to the polymorphic PvMSP-3$\alpha_{359-798}$ during natural infections. Among the best-characterized antigens is MSP-1_{19}, as it is the portion of merozoite surface protein 1 (MSP-1) that remains attached to the merozoite surface during erythrocyte invasion. It is also known to be a target of protective antibodies, which

can mediate inhibition of parasite growth in vitro, providing valuable insights for the development of effective blood-stage vaccines targeting *P. vivax* (49).

Function and role of MSP-1_{19} as of the study PMID: 25889175:

a. **Anchoring and Orientation**:

- MSP-1_{19} helps anchor the larger MSP-1 complex on the surface of the merozoite. **Figure 2.6** shows a schematic representation of the *Plasmodium* merozoite anchorage mechanism.
- While the other fragments are shed, it stays attached throughout the invasion process.
- The correct spatial organization of additional merozoite proteins (such as AMA1, EBA-175, and others) involved in erythrocyte invasion depends on this anchoring.

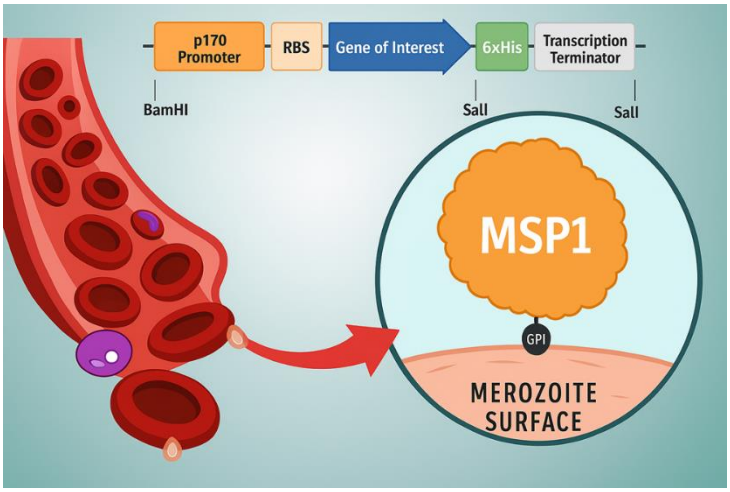


Fig. 2.6: Schematic representation of Plasmodium merozoite invasion: The MSP-1 protein is highlighted on the merozoite surface.

b. **Immune Evasion and Immune Target:**

- Due to its surface exposure and stability, MSP-1_{19} is a prominent target of the host immune response.

- It elicits strong IgG (especially IgG1 and IgG3) antibody responses.
- These antibodies can opsonize merozoites for phagocytosis, block interaction with erythrocytes, indirectly interfering with invasion, and activate complement, leading to merozoite lysis.

c. **Proteolytic Processing & Erythrocyte Invasion:**

- MSP-1_{19} is essential for final processing of MSP-1 just before invasion.
- This cleavage step is critical as it ensures proper detachment of non-essential fragments. It also facilitates tight junction formation between the merozoite and erythrocyte, a key step in invasion.

d. **Structural Stability:**

- The EGF-like domains in MSP-1_{19} provide conformational stability, protecting it from proteolytic degradation.
- This also makes MSP-1_{19} a reliable target for vaccine development, as its structure is less variable compared to other antigens.

Table 2.2: Summary of the mode of action of MSP-1_{19}

Function	Role of MSP-1_{19}
Anchoring	Keeps MSP-1 complex attached to the merozoite membrane
Invasion support	Allows final cleavage and proper invasion machinery assembly
Immune target	Induces protective antibodies (IgG1, IgG3)
Vaccine candidate	Stable and immunogenic structure

Another study in Mangalore (a city in the southern part of India) revealed the measured antibody levels against MSP-1_{19} using enzyme-linked immunosorbent assays (ELISA). Findings showed that 85.4% of *P. vivax* patients and 87.9% of *P. falciparum* patients had

significant levels of anti-MSP-1_{19} antibodies. Anaemia (low haemoglobin levels) and thrombocytopenia (low platelet count) were linked to higher levels of anti-MSP-1_{19} antibodies (42). In vitro studies of the same have provided evidence of how anti-MSP-1_{19} can inhibit the growth of the parasite (44). Since MSP-1_{19} gave such a strong immune response, it becomes a promising candidate vaccine.

2.8. LAB Vector

Lactic acid bacteria (LAB) are a heterologous group of non-sporulating, Gram-positive, microaerophilic, non-mobile, facultatively anaerobic, and catalase-negative microorganisms (35). With a doubling time of 35 to 60 minutes and a fermentative or respiration-type metabolism, this bacterium grows at 30 °C. They are capable of producing lactic acid as a product of carbohydrate fermentation. The Embden–Meyerhof–Parnas (EMP) route allows *L. lactis* to ferment glucose, yielding l (+)-lactic acid as the primary product. Moreover, *lactococci* cannot develop above 6.5% NaCl because they cannot withstand high salt concentrations.

The most well-characterized species of the LAB is *Lactococcus lactis*, which serves as the model organism for the engineering research of this group because of its economic significance. It is generally recognized as safe (GRAS). It has been used safely for a long time in food production because of its probiotic qualities, lack of endotoxins, ability to secrete stable recombinant protein into the growing medium, low protease content, and variety of cloning and inducible expression vectors. The growth of *Lactococci* is rapid, proceeds to high cell densities, and does not require aeration, which also facilitates large-scale fermentation (54).

2.8.1. *E. coli* vs *L. lactis*

Numerous expression systems have been created to produce recombinant proteins; *Escherichia coli* produces the highest protein among prokaryotic systems. The most often employed tactic in this microbe, however, is intracellular protein synthesis (cytoplasm or

periplasm), which necessitates costly and frequently troublesome purifying procedures (36). Additionally, before the intended proteins can be safely given to animals, endotoxins such as lipopolysaccharides (LPS) also need to be eliminated. *L. lactis* is an innovative replacement microorganism for the synthesis of compounds of biotechnological interest, thereby circumventing the issue with bacterial toxins (38). The membrane of *Lactobacillus lactis* contains a variety of lipids, but it is especially abundant in glycolipids and cardiolipin, which are absent from the membranes of *E. coli*. For membrane proteins to be stable, conform, and function, lipids are necessary (46). *L. lactis* is the model LAB and the easiest to manipulate.

The tightly controlled gene expression system known as NICE (Nisin-Inducible Cell Expression) in *Lactococcus lactis* enables the control of overexpressed membrane proteins, making it straightforward to identify and isolate them from crude membrane extracts (47).

Table 2.3: Comparative advantages of *Lactococcus lactis* over *Escherichia coli* as a host for recombinant protein production, highlighting its superior safety profile, secretion capability, and suitability for expressing complex and membrane-associated proteins.

Feature	**Advantage in *Lactococcus lactis***	**Limitations in *Escherichia coli***
Endotoxin-Free Expression	✅ L. lactis naturally lacks lipopolysaccharides (LPS), eliminating the need for expensive and time-consuming endotoxin removal, crucial for therapeutic protein safety.	❌ *E. coli* produces LPS, which must be rigorously removed from protein preparations intended for animal or human use.
Safe for Human Use (GRAS)	✅ Classified as GRAS (Generally Recognized As Safe), making it ideal for	❌ Not GRAS; regulatory hurdles are higher for food or clinical use.

	biopharmaceuticals and food-grade applications.	
Efficient Protein Secretion	✅ Can secrete recombinant proteins directly into the culture medium, simplifying downstream purification and reducing cell lysis steps.	❌ Proteins mostly accumulate intracellularly, requiring cell disruption and complex purification.
Better Folding of Membrane Proteins	✅ Membrane rich in glycolipids and cardiolipin—essential for the correct folding and function of membrane proteins.	❌ Lacks key lipid components, often resulting in misfolded or unstable membrane proteins.
Reduced Inclusion Bodies	✅ Proteins are more likely to remain soluble and functional, with minimal inclusion body formation.	❌ High risk of inclusion body formation requiring denaturation and refolding.
Tightly Controlled Expression (NICE System)	✅ The Nisin-Inducible Controlled Expression (NICE) system allows tight, tunable control over gene expression with minimal leaky expression.	❌ Inducible systems (like IPTG) can leak, causing premature expression or stress on host cells.
Lower Proteolytic Activity	✅ Fewer endogenous proteases help maintain protein integrity during expression.	❌ Higher protease activity can degrade recombinant proteins.
Supports Complex Protein Production	✅ Supports the production of difficult-to-express proteins, including eukaryotic antigens like *Plasmodium* proteins.	❌ Often struggles with complex, disulfide-rich, or eukaryotic proteins due to lack of proper folding environment.

Industrial Scalability & Track Record	✅ Successfully used in up to 3000 L fermentations with stable expression and consistent quality.	✅ Also scalable, but purification and LPS removal remain bottlenecks.

2.8.2. Plasmid

Lactis has shown sustainability for the expression and purification of various strains of *P. falciparum* and is hence used for *P. vivax* as well. The proteins produced as a result of fermentation have shown proper folding, proving recombinant antigens with a range of protein structural complexity can be efficiently expressed in *L. lactis* (39).

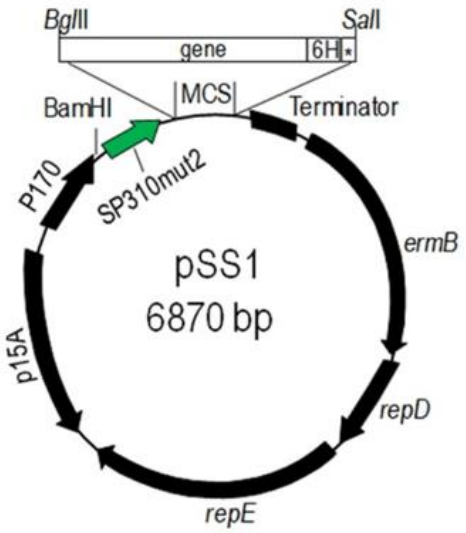


Fig 2.7: Schematic representation of the expression vector PSS1 for *L. lactis* showing structural features including open reading frames.

Cloning of rCMAs and their constituents:

1. p170: lactate-inducible promoter;
2. SP310mut2: secretion signal;
3. MCS: multicloning site;

4. Terminator: transcriptional terminator;
5. ermB: Erythromycin resistance gene;
6. repE; repD: L. lactis replicon and
7. p15A: *Escherichia coli* replicon.

2.9. Strategy:

The process begins with transforming the construct of interest into electrocompetent cells of *L. lactis*, followed by colony screening using SDS-PAGE and ELISA of the best-expressed

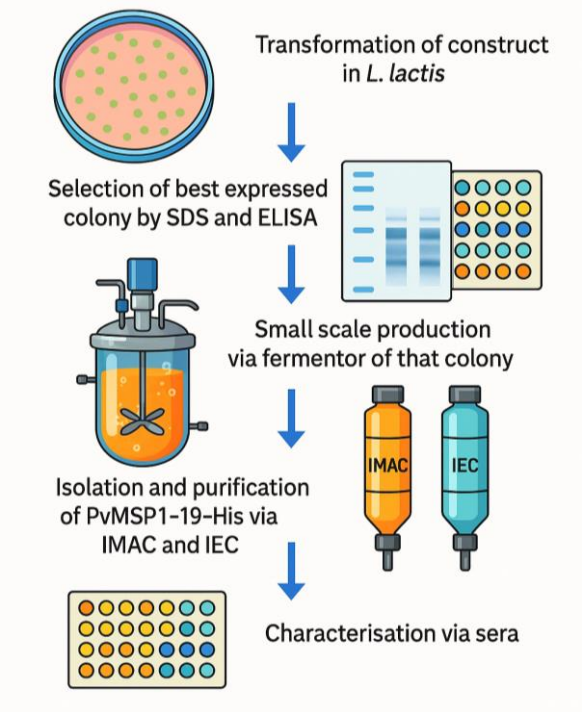

Transformation of construct in L. lactis
Selection of best expressed colony by SDS and ELISA
Small scale production via fermentor of that colony
IMAC
IEC
Isolation and purification of PvMSP1-19-His via IMAC and IEC
Characterisation via sera

Fig. 2.8: Workflow of recombinant PvMSP-1_{19} His-tagged protein production in *Lactococcus lactis*.

colonies. We prepare a fermenter of the best colony and do a small-scale production of the same. The best-expressing clone undergoes small-scale fermentation, after which the protein is purified using immobilized metal affinity chromatography (IMAC) and ion

exchange chromatography (IEC). Final characterization is performed using human sera. The workflow is shown in **Figure 2.8**.

2.10. Future Prospects:

The immune-escaping mechanism of the parasite poses a significant obstacle to the creation of vaccines. A successful blood-stage vaccination needs to trigger memory cells, overcome genetic limitations, and produce humoral and cellular immune responses (52). Even if these blood-stage vaccinations appear promising, it's crucial to remember that there are currently no licensed blood-stage malaria vaccines for general use. RTS, S/AS01 (Mosquirix), and R21/Matrix-M are among the currently authorized malaria vaccines that mainly target the parasite's pre-erythrocytic stage.

Hence, research into blood-stage vaccines is crucial to offer complete protection against malaria by focusing on the multiple phases of the parasite's life cycle. Study on immunogenicity and examine the recombinant MSP-1's folding and structural integrity using X-ray crystallography or circular dichroism (CD) spectroscopy to check for binding to erythrocyte receptors or recognized antibodies.

Future target:

- Chimera vaccine production that targets the potential stages.
- Identification of potential adjuvants that could help in increasing the efficiency of the vaccine.
- Production and transportation costs must be on the lower side.
- Possibility of mass immunisation.

3. Aim and Objective

1. Aim

- To express and produce the protein of interest and validate it as a potential vaccine candidate.

2. Objectives

- To successfully transform the construct of interest in *L. lactis*.
- To select the best-expressed colony and conduct a small-scale production of the same.
- To purify the protein of interest using a His-trap column.
- To characterise our protein of interest.

4. Materials and Methods

4.1. Chemicals Required:

a. Media:

Table 4.1: List of Media

Media	Composition
LB Media	1. LB Powder 2. Sterile water up to the requirement
2X LAB Media	1. MeCit 2. $AmSO_4$ (0.276M) 3. NaAc (1.47M) 4. KPO_4 (1M, pH 7.0) 5. Sterile water up to the requirement
Buffered LAB Medium (Overnight culture media)	1. 2X LAB Media (Half of the required volume) 2. Glucose (5%) 3. Di sodium glycerophosphate (4% w/v) 4. ERM (10µg/mL) 5. Sterile water up to the requirement
Buffered LAB Medium (for Electroporation)	1. 2X LAB Media (Half of the required volume) 2. Glucose (5%) 3. Di sodium glycerophosphate (4% w/v) 4. Sterile water up to the requirement

Fermenter Media	1. 2X LAB Media (Half of the required volume) 2. Glucose (5%) 3. ERM (10µg/mL) 5. Sterile water up to the requirement

b. Buffers:

Table 4.2: List of Buffers

SDS- PAGE	Running Buffer	1. Tris-HCl (0.25M) 2. Glycine (1.92M) 3. SDS (1% w/v)
	Staining Solution	1. Coomasie Brilliant Blue (0.1% w/v) 2. Methanol (40-50% w/v) 3. Glacial Acetic Acid (10% w/v) 4. Distilled Water
	Destaining Solution	1. Methanol (30%) 2. Acetic Acid (10%) 3. Water (70% w/v)
ELISA	Blocking Buffer	5% Skimmed Milk in PBS
	Washing Buffer	PBS with Tween-20 (PBST)
	Substrate Buffer	TMB (3,3',5,5'-Tetramethylbenzidine)

Western Blotting	Running Buffer	1. Tris (25 mM) 2. Glycine (192 mM) 3. SDS (0.1%)
	Transfer Buffer	1. Tris (25 mM) 2. Glycine (192 mM) 3. Methanol (20% v/v) 4. pH around 8.3
	Blocking Buffer	5% Skimmed Milk in TBST
	Washing Buffer	1. Tris (25 mM) 2. NaCl (10% w/v) 3. Tween-20 (0.1%)
	ECL (Detection Buffer)	1. Reagent A: Luminol, a substrate for horseradish peroxidase (HRP), and a buffer, often Tris-HCl. Reagent B: Hydrogen Peroxide
Affinity Chromatography	Binding Buffer (pH 7.2)	1. Tris (50 mM) NaCl (100 mM)
	Elution Buffer (pH 7.2)	1. Tris (50 mM) 2. NaCl (100 mM) Imidazole (1M)
Ion Exchange Chromatography	Binding Buffer (pH 8)	1. Tris (50 mM) 2. NaCl (50 mM) EDTA (1mM)
	Elution Buffer (pH 8)	1. Tris (50 mM) 2. NaCl (1 M) EDTA (1mM)

4.2. Equipment Required:

Table 4.3: List of Equipment

Equipment	Company	Purpose
-80°C Freezer	Eppendorf- Cryo Cube F570 series ULT freezer	Long-term storage of temperature-sensitive biological samples like proteins, DNA, or cells.
Protein Purification System	AKTA Go Protein Purification System from Cytiva	Automated system for purifying proteins using chromatography with a flow rate of 0.01 to 25 mL/min.
Fermenter	Delta Fermenter Biospin 3A	Used for controlled microbial or cell culture growth in large volumes.
Visible Spectrophotometer	LABMAN Scientific Instruments	Measures the absorbance of solutions at visible wavelengths to quantify analytes.
White Light Illuminator	BR Biochem	Provides uniform lighting for visualizing stained gels or blots.
Mini PROTEAN System	BIORAD	Used for SDS-PAGE to separate proteins by size.
Horizontal Electrophoresis Systems	BIORAD	Used for DNA/RNA separation using agarose gel electrophoresis.
Pre-Cast Gel	Invitrogen by Thermo Scientific	Ready-to-use gels for quick and consistent protein electrophoresis.
Vertical Mini Gel Tank	Invitrogen by Thermo Scientific	Apparatus for running mini vertical protein gels.

ChemiDoc	BIORAD, MP Imaging System	High-sensitivity imaging system for visualizing gels, blots, and fluorescence/ chemiluminescence.
NanoDrop Lite Spectrophotometer	Thermo Scientific	Measures nucleic acid or protein concentration from small-volume samples.
Dry Bath Incubator	LABMAN DBI20	Heats samples at precise temperatures in microtubes or vials.
Micro Pulser	BIORAD	Electroporator used to introduce DNA into cells via electric pulses.
Biosafety Cabinet, Class II	BR Biochem	Protects user and samples by providing a sterile environment for biological work.
Multispin	TARSONS	Multi-position centrifuge used for spinning multiple microtubes simultaneously.
Top Filter (0.22µm)	Thermo Scientific/ Sartorius	Sterile filtration of solutions to remove bacteria and particulates.
ELISA Plate Reader	Thermo Scientific Multiskan Sky Microplate Spectrophotometer	Quantifies colorimetric, fluorescent, or luminescent signals in ELISA assays.
pH Meter	Thermo Scientific EUTECH (Handheld Meter Kit)	Measures the acidity or alkalinity (pH) of liquid samples.
Centrifuge	IGENE Labserve	Separates components in liquid samples based on

		density via high-speed spinning.
Mini Centrifuge	SPINWIN MC-00	Compact centrifuge for quick spins and micro-volume sample separation.
Magnetic Stirrer	SPINIT	Uses a rotating magnetic field to mix solutions consistently and evenly.
Weighing Machine	LABMAN Compact Analytical Balance	Precisely measures small quantities of substances for an accurate experimental setup.

4.3. Protocol:

I. Transformation in *E. coli*:

1. Competent *E. coli* cells of the strain DH5α were taken along with the already available plasmid in the lab.
2. DNA containing the plasmid with the construct of interest was taken around 1µl along with a 50µL aliquot of the competent cells.
3. DNA was added to the cell aliquot so that the cells could take up the plasmid.
4. The contents were kept on ice and incubated for 30 minutes.
5. The cells were given a heat-shock treatment, i.e., they were kept in the water bath at 42 °C for 30 seconds and then in ice for 5 minutes.
6. SOC media, also known as super optimum catabolite, is an *E. coli* recovery medium. The culture is supplemented with 250 µL of SOC medium (cells and foreign DNA). The culture was kept in an incubator shaker at 37 °C and 220 rpm for 1 hour.
7. The culture solution is spread on an agar plate for screening.

II. Glycerol Stock Preparation:

1. The culture was incubated at 37° with shaking.
2. 50% glycerol was filled up to 350 µL in 1 mL cryo vials.
3. 650µL of culture was added to it.
4. The culture was homogenized by mixing the contents through thorough pipetting.
5. Vials were put in ice immediately.
6. Once the samples were done, they were frozen at -80° until use.

III. Plasmid Isolation:

1. An overnight culture was prepared by adding 3 mL of LB medium to 3 Falcon tubes of 15 mL each with *E. coli* and were incubated at 37 °C with shaking.
2. The culture was centrifuged at 11000g for 30 seconds. The supernatant was discarded, and the pellet was collected.
3. Kit-based separation: Takara. Machery Nagel Bioanalysis.
4. The pellet was resuspended in 250 µL of the Resuspension Buffer.
5. Lysis Buffer was added and mixed instantly to avoid the buffer settling down. The buffer's contents turn blue.
6. Incubated at room temperature for 2-3 minutes. Contents remain at an acidic pH.
7. The addition of a Neutralizing Buffer made the contents basic. Contents shaken until it becomes colorless.
8. Centrifuged at 11000g for 5 minutes. The pellet was discarded.
9. The column and collector were attached. The supernatant was put in the column provided by the kit. DNA binds with the silica membrane in the column.
10. Centrifuged at 11000g for 5 minutes. The solution in the column was discarded.
11. Column is washed with a Washing Buffer. Centrifuged at 11000g for 1 minute.
12. It was washed again and centrifuged.
13. The contents of the collection tube were discarded. We dry centrifuge the column along with the collection tube. Ethanol was removed in this step.
14. The Elution Buffer was heated around 50°-70°C, then added to the column. Elution was done at 50 µL.

15. The concentration of DNA was checked in a Nanodrop.

- PvMSP-1_{19}- His:

  Concentration: 40.5 ng/µL

  Purity: 1.95

IV. Restriction Digestion:

1. 10 µL of DNA/plasmid was taken in a 0.5µL Eppendorf.
2. To that, 0.5 µL of restriction enzymes BamHI and SalI were added. 2 µL of buffer and 7 µL of nuclease-free water were added, making up to 20 µL.
3. The Eppendorf with the mixture was incubated at 37°C for 2 hours.
4. 0.8g of agarose was dissolved in 100 ml of 1X TAE buffer to make a 0.8% gel.
5. The mixture was heated. The agarose solution was allowed to cool to approximately 50°C or until our hand could rest comfortably on the flask.
6. 5µl of Ethidium Bromide was added.
7. The loading sample is prepared by adding 1 µL of dye to 5 µl the sample.
8. 6µl of the samples (naked plasmid and digested plasmid) and 4 µL marker were loaded in the gel.
9. A voltage of 200 V was applied across the gel.

V. Preparation of electro-competent *L. lactis* cells:

1. *L. Lactis* of the strain LC2 was grown in LAB medium at 30°C for 6-8 hours until an O.D. of 0.5-0.8 at 600nm was obtained.
2. The cells were diluted 100-fold in 25 mL of LAB medium containing sucrose (0.5M) and 0.5% (w/v) Glycine. The culture grew overnight (12-16h) to an OD of 0.7-1.0 at 600 nm.
3. Before harvesting, the cell was cooled down on ice for 10 minutes.

4. Cells by harvested by centrifugation for 10 minutes at 3000g at 4°C and washed twice in 3 ml ice-cold 0.5M sucrose containing 10% (v/v) glycerol.
5. Cells were then suspended in a 7000 µL culture volume of ice-cold 0.5M sucrose containing 10% (v/v) glycerol, preparing the cells for electroporation.
6. The cells were stored in an aliquot (45 µL) at -80°C until needed.

VI. Electroporation of electro-competent *Lactococcus lactis*:

1. Competent cells thawed on ice.
2. In an Eppendorf, 40 µL of cells were mixed with 5 µL of DNA (50-100ng/µL). Then, the mixture was transferred and distributed evenly on the bottom of the cuvette to avoid air bubbles.
3. Pulsed once using the following settings:
    a) Voltage: 1.2 kV
    b) Capacitance: 25 µF
    c) Resistance: 200 Ω
    d) Time constant: 4- 5 ms
4. Immediately following the discharge, 0.95 mL of ice-cold Buffered LAB medium (for electroporation) was added to the cuvette.
5. The mixture was transferred to an Eppendorf tube and incubated at 30°C for 2h.
6. The cell suspension was plated by spreading it on LAB Agar medium containing 1µg/mL erythromycin.
7. The plates were incubated at 30°C for 24-48h to visualise the colonies.

VII. Preparation of overnight culture:

1. 10 Falcon tubes of 15 mL with 3 mL of Buffered LAB media in each were prepared.
2. 10 colonies were selected from the transformed plate.
3. The colony was carefully scraped out from the plate with a pipette tip and released into the tube containing the media. The entire process was carried out in a biosafety cabinet.
4. The tubes were incubated at 30°C for 16 hours.

VIII. Sample preparation:

1. The sample tubes were collected when the solution looked turbid (with some precipitate).
2. The solution was homogenised by thorough pipetting and collected in a 2 mL Eppendorf. Collected the 10 samples similarly.
3. The solution was centrifuged at 6000g for 10 minutes at 4°C.
4. The supernatant was collected in another Eppendorf tube and stored for further use. The pellet was discarded.

IX. SDS gel preparation:

1. The loading sample is prepared by adding 4µL 6X Reducing dye to 20 µL sample.
2. A precast gel was used to check for better visualisation of the best-expressed colonies.

X. ELISA for colonies:

1. Coating: Each well of the ELISA plate was coated with 100 µL of the sample. All the samples were run in duplicates. The samples were incubated overnight at 4°C.
2. Washing: The solution was discarded, and the wells were washed 3 times with PBST buffer to remove unbound antibodies or unbound antigens.
3. Blocking: 5% skimmed milk in 1X PBS buffer was used as a blocking buffer. The plate was incubated for 1 hour. This blocks any unbound sites on the ELISA plate, cleans the plates of any possible unbound antibodies, cuts down the chances of getting false-positive results, and stops nonspecific antibodies from attaching to the plate.
4. Washing: The solution was discarded, and the wells were washed 3 times with PBST buffer.
5. Antibody solution: The primary detection antibody binds directly to the target protein in a direct ELISA. We prepared an anti-His HRP conjugated Antibody solution at a dilution level of 1:3000 in 2.5% skimmed milk in 1X PBST. The plate was put in an ELISA shaker.
6. Washing: The solution was discarded, and the wells were washed 5 times with PBST buffer. This step is crucial for removing unbound antibodies.
7. Colouring Agent: 50 µL of TMB solution was added to each of the desired wells. It was kept for 20 minutes. TMB is the colouring agent, which reacts with HRP to generate a blue colour.
8. Stop Solution: 2N H_2SO_4 was used as the stop solution. We put 25 µL of the stop solution in each well. The acid stops the reaction and produces a yellow colour.
9. Reading: The OD was checked at 450 nm in an ELISA reader.

XI. Fermenter:

1. Inoculum Preparation: A starter culture was prepared by inoculating the best-expressed colony, i.e., colony no. 4, in 3 mL of Buffered Lab media for at least 6-8 hours at 30°C, depending on the growth and its optical density (OD).
2. Media Preparation: Fermenter media was prepared in a sterile condition in the biosafety cabinet.
3. Sterilisation: The fermenter was cleaned, including all related equipment like pumps and tubing.
4. Medium Transfer: The fermenter was filled with the freshly made media.
5. Anaerobic Conditions: Anaerobic conditions were maintained in the fermenter.
6. pH Control: To maintain a steady pH, usually between 6.3-6.9, we set up a pH control system with pumps attached to flasks holding 0.5 N NaOH and 1 M HCl. We set a pH of 6.45.
7. Temperature Control: 30°C was set in the fermenter.
8. Agitation: RPM of 150 of the impeller to guarantee adequate mixing of the culture media.
9. Inoculation: The starter culture was inoculated post-media transfer in the fermenter vessel.
10. Monitoring: Metrics including pH, optical density (OD at 600 nm), and the production of lactic acid or other fermentation products were measured time to time to keep an eye on the fermentation process.
11. Sampling: The samples were collected periodically to analyse cell growth and fermentation products. The OD was recorded to prepare a growth curve for analysis

12. Harvesting: The broth was collected post-fermentation. Using a centrifuge, we harvested the fermentation broth. We centrifuged the culture at 10,000 RPM for 30 minutes.
13. Filter: The supernatant is collected and filtered using a top filter of pore size 0.22 µm.
14. Storage: The supernatant was kept at -20°C for immediate use or at -80°C for long-term storage.

XII. Protein Purification: Immobilised Affinity Chromatography

1. To guarantee an effective purification, the Cytiva AKTA GO System was used for these procedures.
2. The pre-packed Ni-NTA column was attached to the purification system. To remove the storage buffer, it was flushed with five column volumes [CV] of distilled water.
3. The equilibration was done using 5 CV of binding buffer at a flow rate of 1 mL/min
4. The step elution profile used for IMAC purification is displayed below (concentration of imidazole): 0%-3 CV, 35%-2 CV, and 100%-1 CV.
5. To determine whether or not the protein was bound to the column, the sample was run through the column, and the flow-through was collected.
6. The imidazole concentration was increased to elute the bound protein.
    a. Elution 1: 3 CV of 0 mM imidazole
    b. Elution 2: 2 CV of 350 mM imidazole
    c. Elution 3: 1 CV of 1 M imidazole
7. To clean the column, 5–10 mL of distilled water was used at a 2 mL flow rate per minute.

8. To determine at which percentage of imidazole the protein was eluted, an SDS-PAGE gel was run for the selected fractions from the 96-plate fraction collector containing the eluted proteins.
9. After analysing the SDS gel according to the molecular weight marker, the desired portion of the protein was pooled and kept at - 40°C.

XIII. Protein Purification: Ion Exchange Chromatography

1. To guarantee an effective purification, the Cytiva AKTA GO System is used for these procedures.
2. The pre-packed Q-HP column (5 mL) was attached to the purification system. To get rid of the storage buffer, flush the column with five column volumes [CV] of distilled water.
3. At a flow rate of 1 mL/min, equilibrate using 5 CV of binding buffer.
4. The step gradient elution profile used for IEC purification is displayed below (concentration of Sodium Chloride): 0%-5CV, 0-15% - 5CV, 15%-3CV, 15- 30%-5CV, 30%-3CV, 30- 55%-5CV, 55%-3CV, 55-100%- 5CV, 100% -5CV).
5. To determine whether or not the protein is bound to the column, the sample was run through the column, and the flow-through was collected.
6. The NaCl concentration was increased to elute the bound protein.
    a. Elution 1: 5 CV of 0 mM NaCl
    b. Elution 2: 5 CV of 0-150 mM NaCl
    c. Elution 3: 3 CV of 150 mM NaCl
    d. Elution 4: 5 CV of 150-300 mM NaCl
    e. Elution 5: 3 CV of 300 mM NaCl
    f. Elution 6: 5 CV of 300-550 mM NaCl
    g. Elution 7: 3 CV of 550 mM NaCl
    i. Elution 8: 5 CV of 550 mM - 1 M NaCl

c. Elution 9: 5 CV of 1M NaCl

7. 5–10 mL of distilled water was used at a 2/ flow rate per minute to clean the column.
8. To clean the column, 5–10 mL of distilled water was used at a 2 mL flow rate per minute.
9. To determine at which percentage of NaCl the protein was eluted, an SDS-PAGE gel was run for the selected fractions from the 96-plate fraction collector containing the eluted proteins.
10. After analysing the SDS gel according to the molecular weight marker, the desired portion of the final protein was pooled and kept at - 40°C.

XIV. Western Blot:

1. A standard SDS PAGE gel was run until the dye in front runs out of the gel. We extracted the gel by separating the glass plates.
2. Activation: PVDF membrane was soaked in 20% methanol.
3. Pre-soak: The gel extracted was resuspended, filter paper, PVDF membrane, and sponge in transfer buffer.
4. Sandwich cast: A transfer sandwich was prepared as follows:
    a. 2 Sponge
    b. Filter paper
    c. Gel
    d. PVDF membrane
    e. Filter paper
    f. 2 Sponge
5. Transfer: The sandwich cast is moved to the transfer device, which needs to be kept in ice to maintain the temperature of 4°C. After putting the sandwich cast into the device, it was filled with transfer buffer. The electrodes were

positioned over the sandwich cast, making sure that the positive electrode and the gel were separated by the PVDF membrane.

6. Running: A constant voltage of 30V was applied to the apparatus from the power supply for at least 1 hour.
7. Blocking: 5% skimmed milk in TBST was used. Incubation time for blocking should be 1 hour, followed by washing with TBST for 3-5 times.
8. Antibody solution: HRP-conjugated anti-His antibody was used. The solution was prepared at a dilution ratio of 1:5000 in 1% skimmed milk in TBST. The incubation time of the membrane in the Antibody solution should be 1 hour.
9. Development of the blot: ECL substrate was added post-washing of the membrane. Reagent A and Reagent B were coated on the membrane in the ratio of 1:1 (100 µL each).
10. Visualisation: The membrane was visualised in the Chemidoc.

XV. Sera ELISA:

1. The reference buffer was prepared, which is the buffer our protein was eluted to set a blank.
2. Concentration of the pooled protein was checked in a Nanodrop.
   - PvMSP-1_{19}- His:

     Concentration: 0.7 mg/mL
3. Sample preparation: 100 wells were taken into consideration, i.e., 10 mL of sample was required if 100 µL went into each well. If the antigen concentration was taken to be 1 µg/mL, 14.2 µL of the pooled protein was mixed in 10 mL of 1X PBS and vortexed.
4. Coating: Each well of the ELISA plate was coated with 100 µL of the sample. The samples were left overnight at 4°C.

5. Washing: The solution was discarded, and the wells were washed 3 times with PBST buffer to remove unbound antibodies or unbound antigens.
6. Blocking: 300 µL of 5% skimmed milk in 1X PBS buffer was used as a blocking buffer. The plate was incubated for 1 hour. This blocks any unbound sites on the ELISA plate, cleans the plates of any possible unbound antibodies, cuts down the chances of getting false-positive results, and stops nonspecific antibodies from attaching to the plate.
7. Washing: The solution was discarded, and the wells were washed 3 times with PBST buffer.
8. Sera Incubation: 50 µL of different human serum samples were put in the ELISA plate. Samples were run in duplicates. The total number of patient samples taken was 44. The plate was put in an ELISA shaker. Human anti-MSP-1_{19}- IgG binds to our sample.
9. Washing: The solution was discarded, and the wells were washed 3 times with PBST buffer. This step is crucial for removing unbound antibodies.
10. Antibody Solution: In Indirect ELISA, the detection antibody binds to the anti-MSP-1_{19}- IgG that is, in turn, bound to our target antigen. We prepared an anti-Human IgG HRP conjugated Antibody solution at a dilution level of 1:4000 in 2.5% skimmed milk in 1X PBST. The plate was put in an ELISA shaker covered in Aluminium foil.
11. Colouring Agent: 50 µL of TMB solution was added to each of the desired wells. It was kept for 20 minutes. TMB is the colouring agent, which reacts with HRP to generate a blue colour.
12. Stop Solution: 2 N H_2SO_4 was used as the stop solution. We put 25 µL of the stop solution in each well. The acid stops the reaction and produces a yellow colour.
13. Reading: The OD was checked at 450 nm in an ELISA reader.

5. Results and Discussions

Cloning of PvMSP-1_{19} - His:

The plasmid was successfully inserted into *E. coli*. LB media with Erythromycin as an antibiotic marker was used for transformation. The white spots on the plate show the colonies. Isolated colonies were picked **(Figure 5.1)**.

Fig. 5.1: Transformed plate of *E. coli*

Isolation of plasmid containing PvMSP-1_{19} -His:

The plasmid is isolated from *E. coli.* Hence, an agarose gel electrophoresis is performed to reconfirm the cloning process. The restriction enzymes used are BamHI and SalI, for digestion. A successful transformation is confirmed by the appearance of an identifiable band in the lower part of the gel, indicated by the red arrow, suggesting a smaller DNA fragment. The upper bright band is likely plasmid DNA, while the lower clear band at approximately the 750 bp position matches the expected size for the MSP-1_{19} insert (24.8 kDa protein ≈ 744 bp DNA) in Lane 3 **(Figure 5.2).**

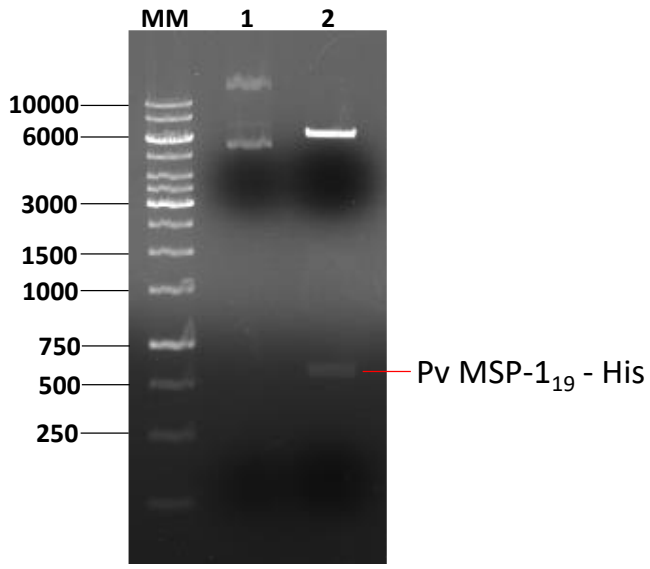


Fig. 5.2: Agarose gel electrophoresis analysis of recombinant plasmid for confirmation.

Lane 1: DNA ladder;

Lane 2: Intact plasmid showing supercoiled, nicked, and linear forms;

Lane 3: BamHI and SalI double-digested plasmid containing the PvMSP-1_{19} insert, releasing the expected fragment size indicative of successful cloning.

Transformation of PvMSP-1_{19} - His in *L. Lactis*:

Competent cells of *L. lactis* were prepared for transformation. The culture solution was spread on an agar plate of LAB media containing ERM post-electroporation. The white spots on the plate show the colonies in **Figure 5.3**. Fewer colonies were seen compared to **Figure 5.1**.

Clear, transparent to white colonies were chosen. Colonies encircled (in **Figure 5.3**) were picked to prepare an overnight culture.

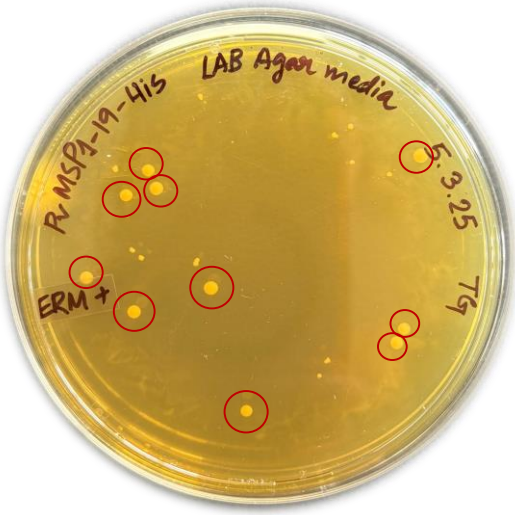


Fig. 5.3: Transformation plate showing colonies of *L. lactis* harbouring PvMSP-1_{19} - His in agar media containing Erythromycin.

Overnight culture of colonies of PvMSP-1_{19} - His:

Ten 15-mL conical tubes (5 mL working volume each) were inoculated with single-colony starters and cultured in Buffered LAB media with ERM at 30 °C.

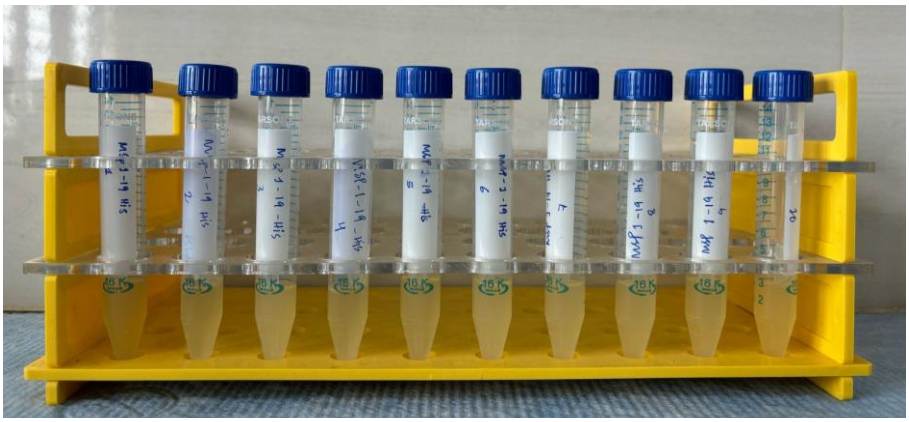


Fig. 5.4: Overnight cultures of 10 selected bacterial colonies of PvMSP - 1_{19} His in Buffered LAB media, prepared in labelled Falcon tubes showing growth.

After about 16 hours, all cultures exhibit the consistent, light-yellow turbidity that characterizes healthy *L. lactis* growth, suggesting similar biomass buildup throughout the panel of recombinant clones (labels 1–10, left–to–right)—no obvious contamination. Upon seeing growth, tubes were photographed on a tabletop rack **(Figure 5.4)**.

A PreCast gel was run to confirm protein expression

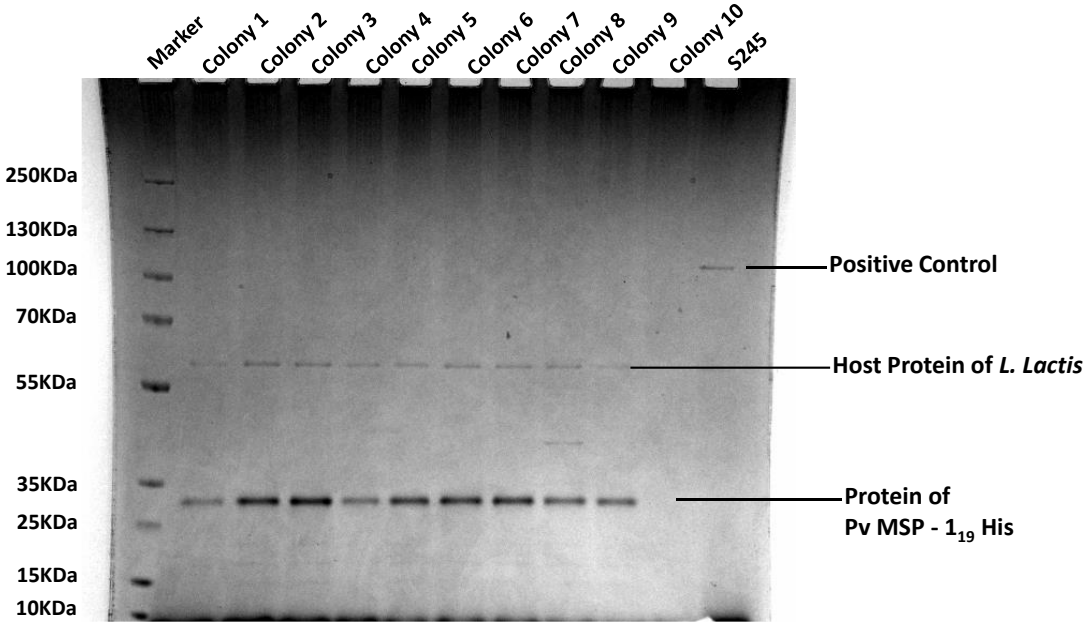


Fig. 5.5: SDS-PAGE analysis of protein expression from overnight cultures of 10 individual *L. lactis* colonies transformed with the recombinant construct.

Here **(Figure 5.5)**, each well corresponds to a different colony. The final lane contains S245, a positive control. The Colony 10 doesn't show any significant expression. A distinct band between 25-35 kDa indicates successful expression of the target protein in multiple clones. The estimated molecular weight of the protein is 24.8 KDa in a 12% handmade gel. The gel is a gradient PreCast gel; hence, the protein settles slightly above.

Identification of Highly Expressing Clones via ELISA:

A direct ELISA was conducted for analysis. The higher absorbance in rows A and B indicates positive antigen-antibody interaction, while low values in row C confirm specificity.

Table. 5.1: ELISA absorbance values at 450 nm for PvMSP-1_{19}-His (rows A and B) compared with negative control (row C, Pro6C C tag).

	1	2	3	4	5	6	7	8	9	10
A	0.211	0.399	0.371	0.465	0.201	0.393	0.35	0.407	0.374	0.055
B	0.172	0.333	0.308	0.353	0.168	0.303	0.336	0.352	0.29	0.051
C	0.082	0.057								

Analysis of the **Table. 5.1**:

- Similar constructs incorporated in various colonies generated notably diverse results, as is common with plasmid-based expression.
- Clones 2, 4, 6, 7, 8 form a clear high-expressing group ($OD_{450} \geq 0.33$; ≥ 5.8-fold over background). Clone 4 is the most promising, showing the highest absorbance (0.465 and 0.353 in duplicate; mean = 0.409).
- The OD values of Clone 10 (and to a lesser extent, Clone 5) are identical to those of the Pro6C C-tag negative control, suggesting that there is little to no recombinant protein present.

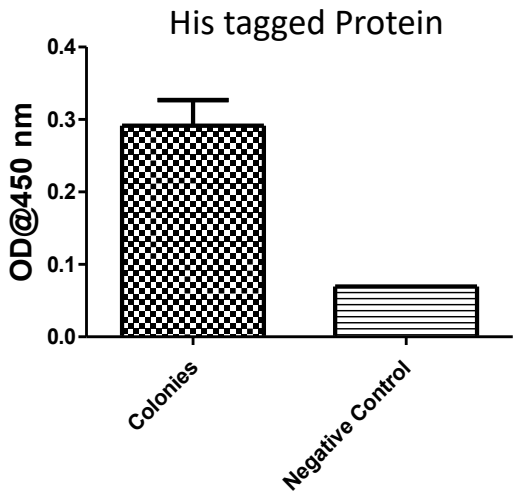


Fig. 5.6: ELISA results showing expression of PvMSP-1_{19}-His in transformed *Lactococcus lactis* colonies.

The bar graph in **Figure. 5.6** represents the average OD@450 nm values of the His tagged protein in the selected colonies compared to the negative control. It shows individual colonies responding against the anti-His antibody, indicating significantly higher antigen expression in recombinant colonies relative to the control.

Optimization of Small-Scale Fermentation for PvMSP-1_{19} - His Production:

Starter culture was incubated 6-8 hours before the fermenter was set up. A 16-h, 1-L Batch culture at 30 °C, with pH held at 6.45 via automatic NaOH addition. The pictures in **Figure 5.7** depict a 2 L glass stirred-tank bioreactor system used to cultivate recombinant *Lactococcus lactis* that expresses PvMSP-1_{19}-His.

Figure 5.7 (i): Fermentation Start (0 hours):

Sterile Fermenter Media (refer to **Table No. 4.1**) supplemented with ERM in the bioreactor. The composition of Fermenter Media gives the media a clear to slightly

brownish appearance. There is no discernible cell density. The system is used under complete sterile connections,

Fig 5.7 (i)

Fig 5.7 (ii)

Fig 5.7: Fermenter Setup: *Lactococcus lactis* Cultivation Before and After 16 Hours of Growth

for temperature control, alkali (NaOH) addition, pH probes, aeration, and sampling.

Figure 5.7 (ii): End of Fermentation (16h):

The culture became opaque and dense after 16 hours at 30°C, indicating significant growth of *L. lactis*. The buildup of bacterial biomass is what causes the turbidity. Automated NaOH addition was used to keep the pH close to 6.8-7 to offset the production of lactic acid during active metabolism. This change from a clear medium to a dense, turbid culture visually confirms successful biomass

accumulation and suggests good conditions for downstream protein purification (IMAC).

Microscopic Validation of Lactococcus lactis Morphology:

The Gram-stained image **(Figure 5.8)** displays the characteristic morphology of *Lactococcus lactis*, which is distinguished by spherical, Gram-positive cocci that are generally arranged in short chains or tiny clusters. Because of a thick layer of peptidoglycan on their cell walls, which holds the crystal violet stain in place even after the decolorization process, gram-positive organisms are distinguished by their bright purple tint. The observed clustering pattern is consistent with the known growth behaviour of *L. lactis* in fermentation conditions that are rich in nutrients. This confirms the authenticity and purity of the culture for recombinant protein expression.

Furthermore, low contamination is indicated by the constant staining and absence of other morphological types, which is crucial for later processes like protein purification.

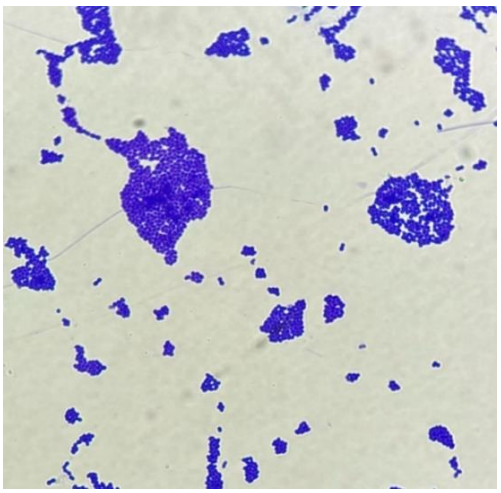


Fig. 5.8: Gram-stained image of *Lactococcus lactis* under oil immersion (100x magnification).

Purification of PvMSP-1_{19} - His via Immobilised Affinity Chromatography:

Immobilized-metal affinity chromatography (IMAC) is a protein-purification method that uses a unique interaction between transition-metal ions that are immobilized on a solid matrix and electron-donating groups on a protein, most frequently the imidazole rings of histidines.

Here Ni^{2+}-NTA is the column used for this His-tagged protein. While the His-tagged protein stays bound, non-specifically bound proteins are removed by low imidazole concentrations (or mild salt/pH changes).

Sample loaded: 500 mL *L. lactis* culture supernatant, clarified and filtered.

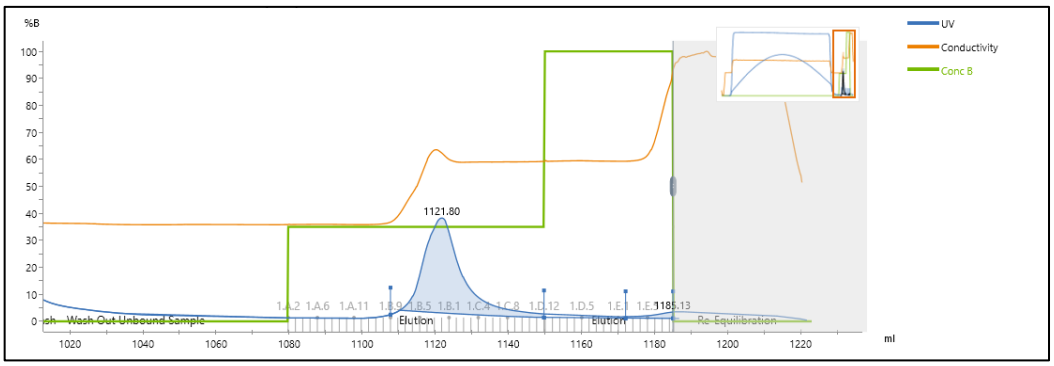


Fig 5.9: IMAC purification chromatogram of His-tagged PvMSP - 1_{19} protein using ÄKTA system.

The UV absorbance in **Figure 5.9** at 280 nm (blue line) indicates the protein elution profile, while the green line represents the imidazole gradient (Conc. B), and the orange line shows conductivity (mS/cm).

Peak at ~1121.8 mL represents the major eluted target protein with 96.42% of total peak area, suggesting successful purification at 35% (350 mM) concentration of imidazole.

Peak at ~1185.1 mL likely represents minor contaminants or late-eluting species. Fractions collected across the elution were analysed by SDS-PAGE to confirm purity.

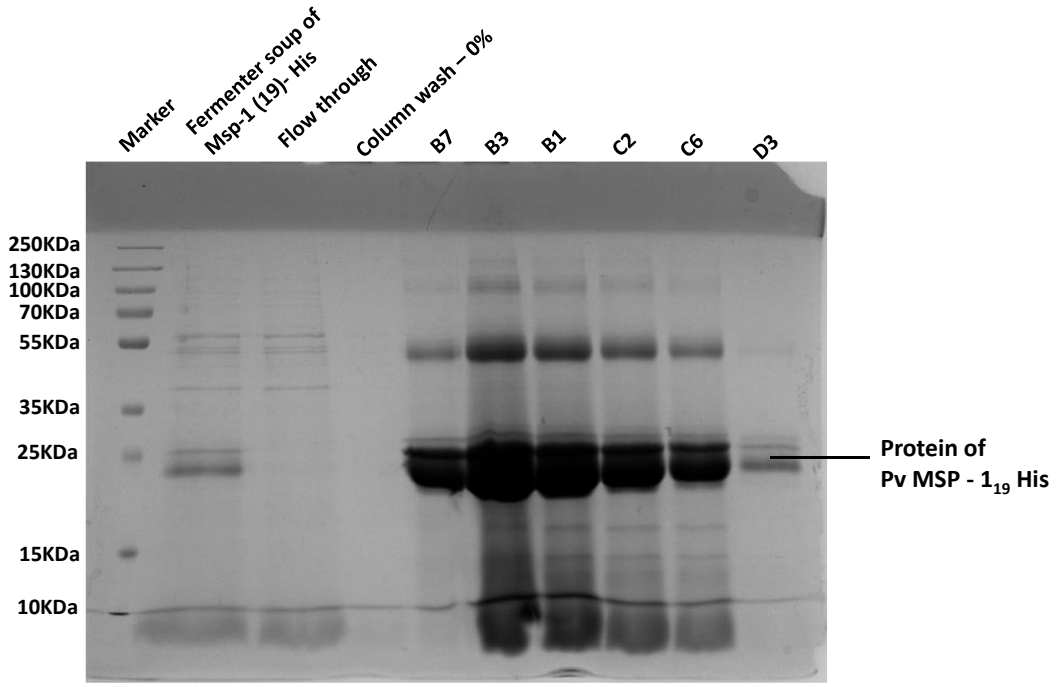


Fig. 5.10: SDS-PAGE analysis of protein purification by Immobilized Metal Affinity Chromatography (IMAC).

A 12% polyacrylamide gel, as in **Figure 5.10,** was used to assess purification steps. A prominent band at ~25 kDa (indicated by the arrow) corresponds to the purified target protein. This band's intensity across several elution fractions indicates that IMAC purification was successful. The strong signal implied a high concentration of the target protein in the eluted fractions.

Enhanced Purification of PvMSP-1_{19} - His through Ion Exchange Chromatography:

Ion exchange chromatography (IEC) is a potent method for separating proteins, peptides, or other charged biomolecules according to their net surface charge. It functions by attaching molecules to a resin that is either negatively charged (cation exchange) or positively charged (anion exchange). Proteins are loaded onto the column in a way that causes them to bind according to their charge. The ionic interactions between the protein and the resin are then broken by progressively raising the salt concentration or altering the pH, which elutes (washes them off).

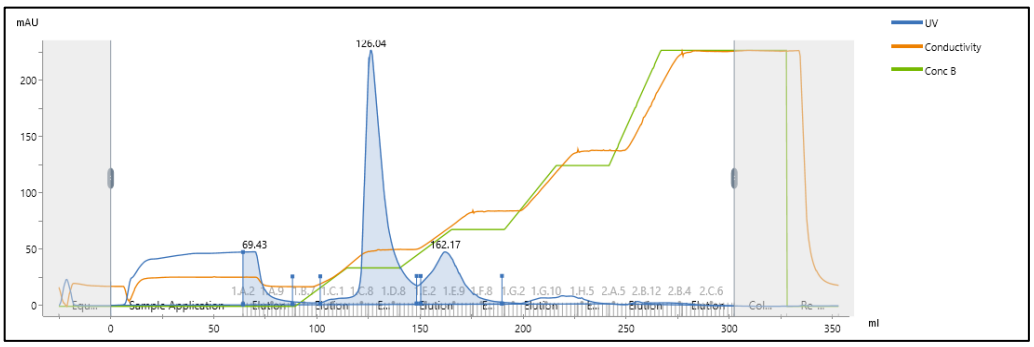


Fig. 5.11: IEC purification chromatogram of His-tagged PvMSP-1_{19} protein using ÄKTA system by gradient elution.

The UV absorbance (blue line) indicates protein elution in **Figure 5.11**, with three distinct peaks (A, B, and C) at retention volumes of 69.43 mL, 126.03 mL, and 162.17 mL, respectively. Peak at ~126.04 mL represents the major protein fraction with the highest UV absorbance and area percentage (66.2%). The conductivity (orange line) and buffer B concentration (green line) profiles reflect the salt gradient used during elution. Data were acquired using Cytiva UNICORN software.

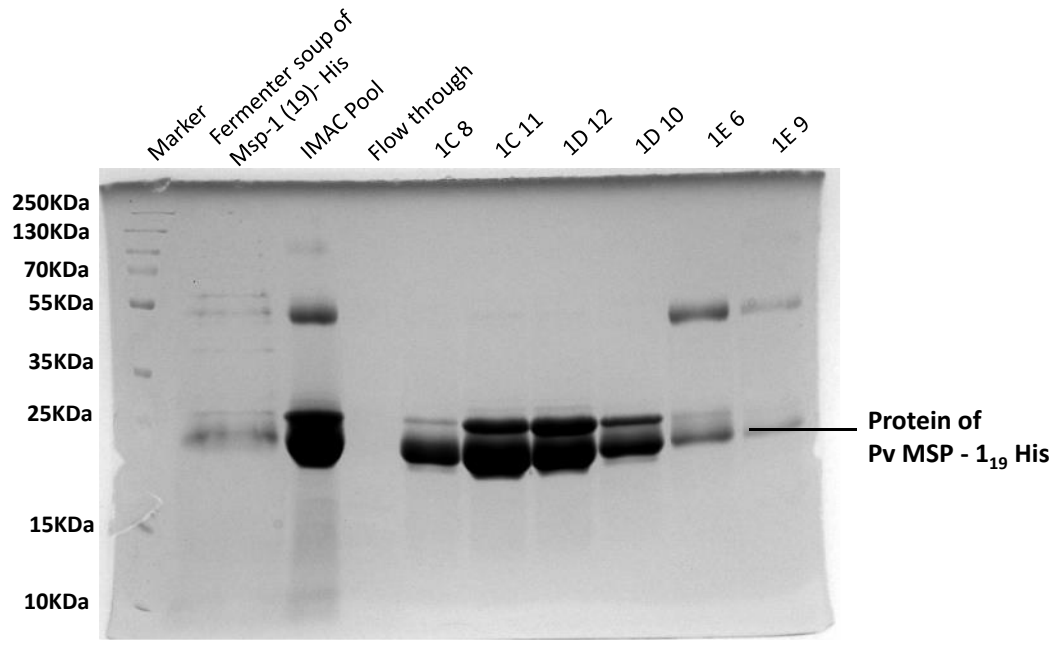


Fig. 5.12: SDS-PAGE analysis of protein purification using Ion Exchange Chromatography (IEC).

A 12% polyacrylamide gel, as in **Figure 5.12,** was used to monitor purification steps. A prominent band at ~25 kDa (indicated by the arrow) represents the target protein, which is enriched in elution fractions 1C11 and 1C12.

Western Blot Analysis for Confirmation of His-tagged PvMSP-1$_{19}$ - His Expression:

Coomassie-stained SDS PAGE and Western blots were prepared **(Figure 5.13)** with the available samples of the protein. Samples of proteins from various phases of expression and purification have been analysed with both reducing and non-reducing dye and compared.

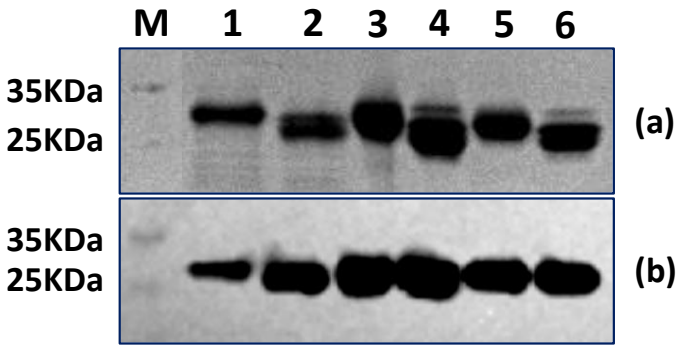


Fig 5.13: SDS-PAGE and Western blot analysis of recombinant PvMSP-1_{19} His expression and purification from *Lactococcus lactis*.

- Lane M: Marker
- Lane 1: Fermenter soup (+)
- Lane 2: Fermenter soup (-)
- Lane 3: IMAC Pool (+)
- Lane 4: IMAC Pool (-)
- Lane 5: IEC Pool (+)
- Lane 6: IEC Pool (-)

Figure 5.13(a): The induced samples of Lanes 1, 3, and 5 show clear bands of samples with DTT, near the predicted size of PvMSP-1_{19}-His. Lanes 1, 3, and 5 contain samples without DTT addition, hence they appear a little below.

Figure 5.13(b): Matching samples probed with an anti-His monoclonal antibody in a Western blot. The presence of the His-tagged recombinant protein is confirmed by the detection of a particular band exclusively in the induced culture (Lane 2) and its purified fractions using IMAC (Lane 4) and IEC (Lane 6).

Serological Assessment of Recombinant PvMSP-1_{19} - His Using Human Sera:

Human serum samples were taken along with our protein to conduct an indirect ELISA. The antigens (protein of interest) were immobilised so that anti- MSP-1_{19}-IgG binds to our antigen from the serum. HRP-conjugated anti-His antibodies were used as Secondary antibodies. The OD was checked at 450nm and recorded. Elevated OD values in a subset of samples suggested immunological recognition of the antigen or previous exposure, indicating seropositivity to PvMSP-1_{19}-His as shown in **Figure 5.14**.

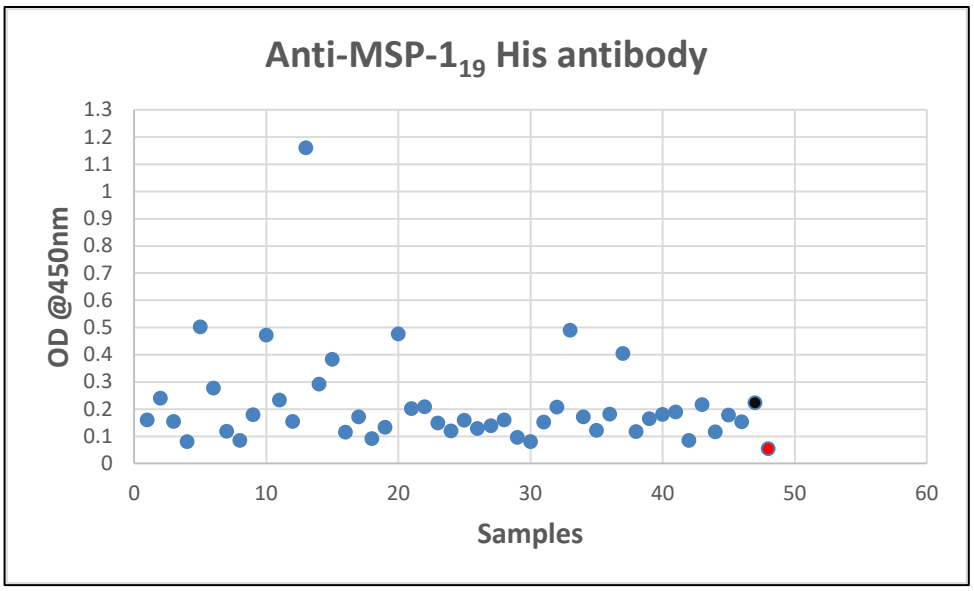


Fig 5.14: Serological reactivity of human samples against recombinant PvMSP-1_{19}-His protein.

a. Black Dot - Libarian pool
b. Red dot- Secondary control
c. Blue dots- All patient samples

Indirect ELISA was used to screen 50 distinct human serum samples that were already infected with malaria for the existence of anti-MSP-1_{19} IgG antibodies.

Analysis of the Sample:

i. Positive Signals: High OD values, like those of samples 33 (OD: 0.4905) and 37 (OD: 0.404), suggest a positive serological response. This could be due to immunological exposure to the PvMSP-1_{19} antigen or a history of malaria.

ii. Moderate Reactivity: Samples 32, 36, 39, and 40 may be classified as mildly positive or slightly reactive based on the cutoff criteria because of their mid-range OD values (about 0.2–0.4).

The recombinant protein was being investigated as a vaccine antigen, its immunogenicity has been evaluated in population members exposed to *P. vivax*. It was tested whether antibodies present in naturally infected individuals recognize the recombinant PvMSP-1_{19} protein. It validates the production and purifying processes by confirming that the produced and purified PvMSP-1_{19}-His protein maintains antigenicity, or the ability to bind antibodies.

PvMSP-1_{19} - His Protein Characterisation:

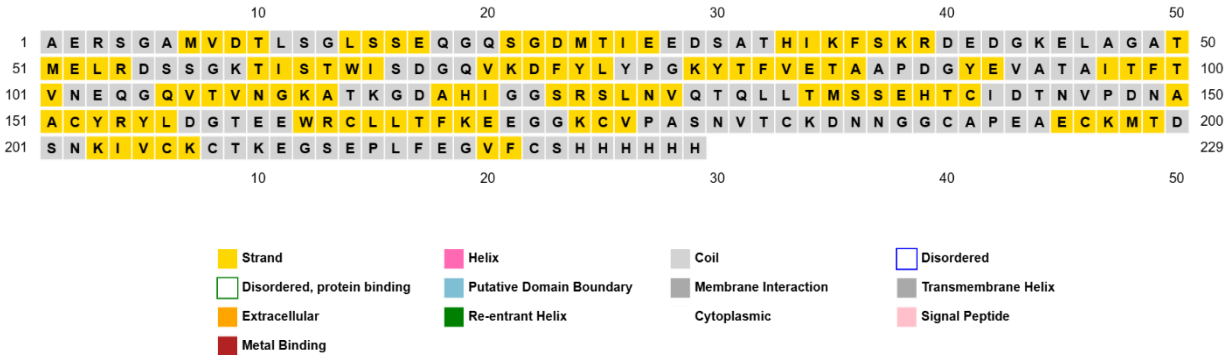

10 20 30 40 50
1 A E R S G A M V D T L S G L S S E Q G Q S G D M T I E E D S A T H I K F S K R D E D G K E L A G A T 50
51 M E L R D S S G K T I S T W I S D G Q V K D F Y L Y P G K Y T F V E T A A P D G Y E V A T A I T F T 100
101 V N E Q G Q V T V N G K A T K G D A H I G G S R S L N V Q T Q L L T M S S E H T C I D T N V P D N A 150
151 A C Y R Y L D G T E E W R C L L T F K E E G G K C V P A S N V T C K D N N G G C A P E A E C K M T D 200
201 S N K I V C K C T K E G S E P L F E G V F C S H H H H H H 229
10 20 30 40 50
Strand
Helix
Coil
Disordered
Disordered, protein binding
Putative Domain Boundary
Membrane Interaction
Transmembrane Helix
Extracellular
Re-entrant Helix
Cytoplasmic
Signal Peptide
Metal Binding

Fig. 5.15: The recombinant PvMSP-1_{19}-His protein's amino acid sequence highlighted. Color-coding of residues denotes their structural or functional classification.
(The 2D model was predicted by using PSIPRED).

Data derived from Expasy ProtParam about PvMSP-1_{19}-His:

i. Number of amino acids: 229

ii. Molecular weight: 24819.39

iii. Theoretical pI: 5.01

iv. Total number of negatively charged residues (Asp + Glu): 35

v. Total number of positively charged residues (Arg + Lys): 21

vi. Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm, measured in water.

- Ext. coefficient 20565

  Abs 0.1% (=1 g/l) 0.829, assuming all pairs of Cys residues form cystines

- Ext. coefficient 19940

  Abs 0.1% (=1 g/l) 0.803, assuming all Cys residues are reduced.

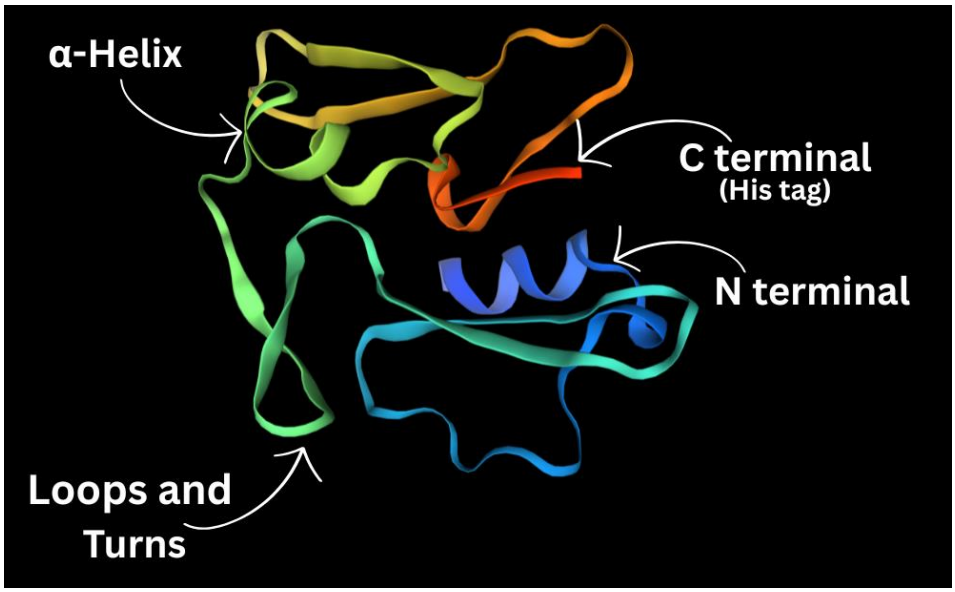


Fig 5.16: Ribbon diagram of the PvMSP-1_{19}-His protein structure predicted. The α-helices, β-strands, and connecting loops are depicted. The N-terminus and C-terminus are labeled, and the His-tag is shown in red. This visualization was generated using

With a calculated molecular weight of roughly 24.8kDa, the recombinant protein described in this study has 229 amino acid residues. The molecular weight is within an acceptable range for expression in heterologous systems, like the host used in this investigation, *Lactococcus lactis*. The protein's relatively small size for recombinant expression is beneficial because it may lower the chance of inclusion body formation and promote appropriate folding.

The protein has a net negative charge at physiological pH (7.0–7.4), according to its theoretical isoelectric point (pI), which was determined to be 5.01. Low pI proteins are typically more soluble in mildly basic environments, which is consistent with the typical buffer systems utilized in affinity chromatography.

Also at 280 nm, the extinction coefficients under oxidizing and reducing conditions were 20565 $M^{-1}cm^{-1}$ and 19940 $M^{-1}cm^{-1}$, respectively. According to these values, the protein has a moderate amount of cysteine residues and several aromatic residues, most likely tyrosine and tryptophan. It makes the protein a potent vaccine antigen, as appropriate folding and epitope presentation are essential for generating a strong immune response.

6. Conclusion

Malaria is one of the most serious public health issues in the tropics and subtropics. This investigation successfully establishes the heterologous expression of the *Plasmodium vivax* merozoite surface protein 1 C-terminal 19-kDa fragment (PvMSP-1_{19}-His), a blood stage antigen, in the GRAS categorised host *Lactococcus lactis*, leveraging the pSS1 expression vector under the control of the nisin-inducible p170 promoter. Molecular cloning was validated via restriction endonuclease analysis, and expression was confirmed through SDS-PAGE and Direct ELISA using anti-His antibodies, affirming the fidelity of recombinant protein production.

The best expressed clone was inoculated in a fermenter, and the protein was subsequently isolated using immobilized metal affinity chromatography (IMAC) and further refined by ion-exchange chromatography (IEC), yielding a protein preparation of appreciable purity. The western blot confirms the presence of His tagged proteins in the isolated samples. Importantly, the recombinant protein demonstrated specific immunoreactivity with human sera, confirming its structural fidelity and potential utility in serological assays or as a vaccine antigen, indicating its suitability for downstream immunological applications. The ability of *L. lactis* to express a eukaryotic protein such as PvMSP-1_{19} supports its promise as a GRAS-certified expression platform for antigen production, particularly for oral or mucosal delivery applications.

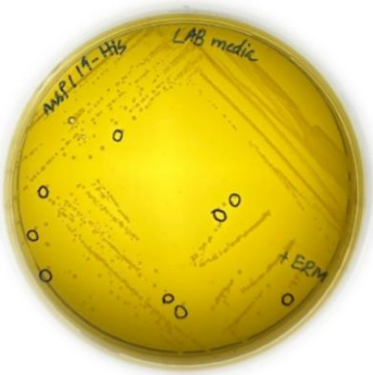
This study affirms the feasibility of expressing PvMSP-1_{19}-His in a cost-effective, scalable microbial platform and advances the broader pursuit of a subunit vaccine against *P. vivax*. The expression system and workflow established herein offer a promising foundation for translational applications in both vaccine development and serodiagnostic innovation in the global fight against malaria.

7. Appendix

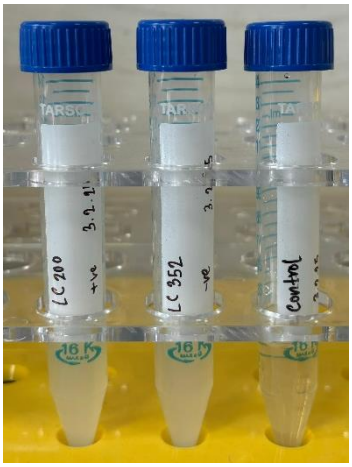
a. Amino acid sequence used in study: His tagged (N-C terminal)

AERSGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTIST
WISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIGGSRS
LNVQTQLLTMSSEHTCIDTNVPDNAACYRYLDGTEEWRCLLTFKEEGGKCVPASNVTCKDNN
GGCAPEAECKMTDSNKIVCKCTKEGSEPLFEGVFCSHHHHHH

b. Streaked Plate of PvMSP-1 $_{19}$ - His



c. Overnight cultures of positive, negative and a control to compare



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Government of India

Date: 20/05/2025

This is to certify that the thesis titled: "Production and Characterisation of Recombinant *Plasmodium vivax* Merozoite Surface Protein-1_{19} Expressed in *Lactococcus lactis*" submitted by Tiasha Ghosh, a student of MSc. in Biotechnology at Sister Nivedita University, Kolkata, has been reviewed for academic integrity and originality. The work was carried out under my supervision and, to the best of my knowledge, has plagiarism within 10%. Proper citations and acknowledgements have been provided wherever necessary. The contents are the original intellectual effort of the student, and any sources used have been appropriately referenced.

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Text-Only Report
Production and Characterisation of Recombinant Plasmodium vivax Merozoite Surface Protein-1$_{19}$ Expressed in Lactococcus lactis
Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of
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